REMARKS

Claims 1-17 and 21-26 are pending. Claims 2, 6 and 15 have been amended. Applicants respectfully request entry of the amendments, which merely deal with correction of typographical errors, or to clarify the invention. No new matter has been added. Applicants respectfully request reconsideration of the present rejections in view of the present remarks.

Rejections under 35 U.S.C. § 102

The Office rejected claims 1-5, 7-10 and 13 under 35 U.S.C. 102(b) as allegedly being anticipated by Okihara *et al.* (J. Macromol. Sci. Phy. (1991) B30, 119-140). In particular, the Office indicated that "[p]olymers of lactic acid and glycolic acid are water-soluble." (Office Action, page 2). Applicants must respectfully disagree.

As indicated in the response to the previous Office action, the Okihara reference is silent regarding water-soluble or water dispersible polymers. The Okihara reference only describes non-substituted homopolymers, particularly the homopolymers of L-lactic acid and D-lactic acid which are crystalline polymers and mostly insoluble in water. A "homopolymer" is a polymer having a chain structure in which all building units are of the same type. While lactic acid is recited in claim 2, it is recited as a substituent, not as the water soluble or water dispersible polymer itself.

Furthermore, as indicated in the Declaration of Professor Hennink ("Hennink Decl." attached as Exhibit 1), who is a co-inventor in the present application, "[p]oly(lactic acid) and its copolymers with glycolic acid are not soluble in water, but they only dissolve in organic solvents. . . . The degradation products may be water-soluble. The polymer itself is not." (See Hennink Decl., ¶ 6; see also van Nostrum *et al.*, ACS Symposium Series 2003, 846: 129-141, attached as Exhibit 2, particularly on page 133, lines 7-9). Further, as indicated in the Hennink Decl., no water is present in the homopolymer system described in Okihara at all. Thus, "it is impossible to have a hydrogel present." (Hennink Decl., ¶ 5).

Because the Okihara reference fails to describe hydrogel compositions, the claims are not anticipated. Thus, Applicants submit that the claims as amended are novel, and respectfully request that this rejection be withdrawn.

The Office also rejected claims 1-10, 14 and 21-26 under 35 U.S.C. § 102(b), as allegedly being anticipated by Hennink *et al.* (WO 98/00170). As the Office has indicated, Hennink discloses hydrogels containing hydrolysable bonds consisting of two interpenetrating networks. The Office also indicated that "Hennink does not exclude oligomers that are formed from the d- and l- monomers." (Office Action, page 3).

Whether or not WO 98/00170 excludes oligomers formed from d- and l- monomers is irrelevant. As indicated in the Hennink Decl. ¶ 8-9, WO 98/00170 does not disclose a hydrogel comprising two polymers, each having a chiral substituent that is in essence complementary to each other. The only reference made to the isomeric form of the lactide is in Example 3, which describes the synthesis of dex-lactate-HEMA by coupling L-lactide and HEMA thereby forming HEMA-lactate, and coupling the HEMA-lactate to dextran. In this example, the lactate is only used as a hydrolysable spacer, and not as a chiral substituent of a water soluble polymer that interacts noncovalently with another polymer having a lactide substituent of opposite chirality. The hydrogels in WO 98/00170 are prepared by free radical polymerization of a crosslinkable group such as methacrylate, acrylate, vinyl ethers and vinyl esters, thereby forming a covalent interaction between the crosslinkable groups. (See e.g., WO 98/00170 on 6:23-27; 9:14-23; and Example 5).

However, crosslinking of polymers to form hydrogels by chemical means has the disadvantage that the required conditions may affect the drugs encapsulated within the hydrogel. Thus, network formation by physical non-covalent interactions between groups or segments of polymers is favored. To create physical cross-links, dextran was substituted with oligolactic acid side chains containing L-lactic acid (dex-L-OLA) or D-lactic acid (dex-D-OLA) enantiomers. Surprisingly, when aqueous solutions of dex-L-OLA and dex-D-OLA with minimum chain lengths of 11 lactic acid units are mixed, the L-OLA and D-OLA grafts associate to form a hydrogel. (See Hennink *et al.*, "Fast Biodegradable polymers," attached at Exhibit 1B, pages 411-12).

Thus, unlike the hydrogels in WO 98/00170, the invention as claimed relates to a hydrogel comprising two polymers, each having a chiral substituent that is in essence complementary to each other. Because Hennink does not describe hydrogels comprising two polymers having chiral substituents that are in essence complementary to each other, wherein the groups on the polymers interact noncovalently, the invention as claimed is novel. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Rejections under 35 U.S.C. § 103

The Office rejected claim 11 under 35 U.S.C. § 103(a), as allegedly being unpatentable under Hennink (WO 98/00170). Claim 11 depends from claim 1, and contains all the limitations in claim 1. As previously indicated, the Hennink reference neither teaches nor suggests hydrogels comprising two polymers having chiral substituents that are in essence complementary to each other, wherein the groups on the polymers interact noncovalently. Thus, claim 11 is nonobvious under Hennink and Applicants respectfully request that this rejection be withdrawn.

The Office also rejected claim 12 under 35 U.S.C. § 103(a), as allegedly being unpatentable under Okihara. Claim 12 depends from claim 1, and contains all the limitations in claim 1. As previously indicated, the Okihara reference fails to describe hydrogels at all. Thus, claim 12 is nonobvious under Okihara and Applicants respectfully request that this rejection be withdrawn.

Furthermore, the Office rejected claims 15-17 under 35 U.S.C. § 103(a), as allegedly being unpatentable under De Jong *et al.* (Macromolecules 31:6397-6402 (1998)), in view of Brannon-Peppas (Int. J. Pharm. 116: 1-9 (1995)). In particular, the Office indicated that "[t]he lactides and glycolides are water-soluble and thus De Jong and Brannon both disclose waster-soluble [sic] polymers." (Office Action, pages 4-5). Applicants must respectfully disagree.

De Jong teaches the synthesis of lactic acid oligomers from heating a neat mixture of lactide and a compound with a primary hydroxyl group such as 2-(2-methoxyethoxy)ethanol as initiator, and subsequent addition of stannous octoate as catalyst. In particular, a mixture of L-

lactide (or D-lactide) was stirred at 130 °C "until the lactide was molten." (See, De Jong *et al.*, page 6399 at column 1). De Jong is silent regarding water soluble or water dispersible polymers, let mixing two mixtures of water soluble or water dispersible polymers, each substituted with oligomers or co-oligomers at least partly formed from chiral monomers of opposite chirality.

Furthermore, De Jong fails to describe an aqueous system. As previously indicated, poly(lactic acid) and its copolymers with glycolic acid are insoluble in water. De Jong's failure to teach the preparation of hydrogels as claimed is not remedied by Brannon-Pappas, which merely describes the use of biodegradable polymers in controlled drug delivery. Thus, even if De Jong and Brannon-Pappas were combined, the combination fails to teach the process as claimed. Accordingly, claims 15-17 are nonobvious under De Jong, in view of Brannon-Pappas, and Applicants respectfully request that this rejection be withdrawn.

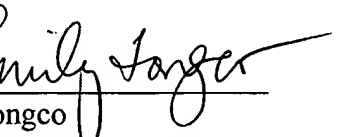
CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 313632001000. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: January 20, 2005

Respectfully submitted,

By 

Emily C. Tongco
Registration No.: 46,473
MORRISON & FOERSTER LLP
3811 Valley Centre Drive, Suite 500
San Diego, California 92130
(858) 314-5413

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Wilhelmus Everhardus Hennink, Cornelus Franciscus van Nostrum, and Silvia Johanna de Jong

Examiner: B. Fubara

Confirmation No.: 8024

Group Art Unit: 1615

Serial No.: 09/913,967

Docket: 313632001000

Filed: December 31, 2001

Dated: January 20, 2005

For: Stereocomplex hydrogels

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I hereby certify this correspondence is being deposited with the United States Postal Service as first class mail, postpaid in an envelope, addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450
on January 20, 2005
Signature:

DECLARATION UNDER 37 CFR 1.132

I Wilhelmus Everhardus Hennink of Zuidplaslaan 120 NL-2743 CZ Waddinxveen, the Netherlands declare as follows.

1. I am employed by the University of Utrecht as professor. I am an expert in the field of hydrogel compositions, as evidenced by the *curriculum vitae* attached hereto as Exhibit A. I am an inventor in the present application and am also a co-inventor in WO 98/00170

2. I have reviewed the Office Action mailed by the Examiner on October 20, 2004, and the references cited by the Examiner in the Office Action. This declaration is being submitted in response to the Office Action to distinguish the present invention from the prior art references.

3. In accordance with the invention, a hydrogel is formed by mixing aqueous solutions or dispersing of a water-soluble or dispersible polymer (e.g. dextran) to which oligomers of opposite chirality are grafted or otherwise substituted. By this process a physically linked hydrogel is obtained.
Stereocomplex formation between poly(D-lactic acid) and poly(L-lactic acid) has been demonstrated in the prior art to occur when these polymers are dissolved in a suitable organic solvent (e.g. dichloromethane) followed by evaporation of the solvent.
We were the first who demonstrated that stereocomplexes can be formed by mixing aqueous solutions/dispersions of a water-soluble or dispersible polymer (e.g. dextran) to which oligomers of opposite chirality are substituted (preferably grafted).

4. One of the important aspects distinguishing the subject-matter of present claims from the cited references of Okihara et al. (J. Macromol. Sci. Phys (1990) B30 (1 & 2) 119-140) and WO 98/00170 (in the name of the undersigned) is that the present claims relate to a hydrogel composition comprised of a mixture of two types of water soluble or water dispersible polymers that are substituted with oligomers or co-oligomers, wherein the (co-)oligomers in the first polymer are at least partly formed from chiral monomers and wherein the (co-)oligomers in the second polymer are at least partly formed from chiral monomers with a chirality that is opposite to that of said monomers in the first polymer, such that the chiral part of the (co-)oligomers are in essence complementary to that in the first polymer.

5. Okihara et al. does not disclose a hydrogel. Firstly, no water is present in the described system. Hence, it is impossible to have a hydrogel present.

6. Secondly, Okihara et al. does not disclose water soluble polymers. The Examiner states (on page 2 and also other pages) that "polymers of lactic acid and glycolic acid are water-soluble; at the worst they are sparingly soluble". This statement is incorrect. Poly(lactic acid) and its copolymers with glycolic acid are not soluble in water, but they only

dissolve in organic solvents. Confusingly, these polymers are sometimes called water-soluble in literature, but this characteristic refers to the ability of these polymers to degrade to low molecular weight degradation products when solid specimens of these polymers are placed in an aqueous solution. The degradation products may be water-soluble. The polymer itself is not. This degradation process takes from a few weeks to a years, depending on a great number of factors) (see e.g. Hennink WE, Van Steenis, JH and Van Nostrum CF. Fast degradable polymers. In: Reflexive Polymers and Hydrogels. In: Understanding and designing fast responsive polymeric system. Ed: Yui, N. Mrsny RJ, and Park K. CRC Press page 401-423, 2004). This alleged "water-solubility" is something completely different from the physico-chemical definition of solubility (i.e. the generally used definition).

7. Thirdly, Okihara et al. does not disclose polymers substituted with an oligomer. Okihara et al. discloses non-substituted homopolymers.

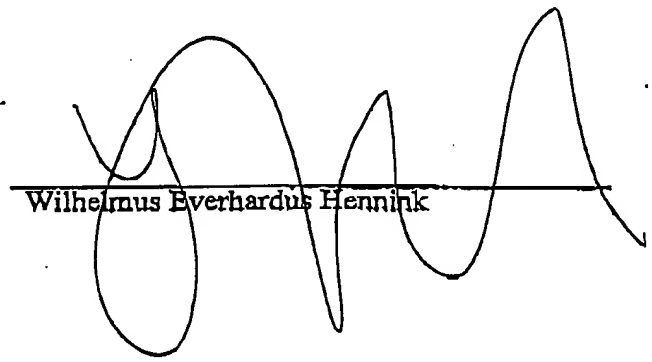
8. WO 98/00170 does not disclose a hydrogel comprising two different substituted polymers, wherein the chiral parts of the substituents are in essence complementary to each other either.

9. The only reference made to the isomeric form of the lactide is in Example 3, which describes the synthesis of dex-lactate-HEMA by coupling L-lactide and HEMA thereby forming HEMA-lactate, and coupoling the HEMA-lactate to dextran. In this example the lactate is used as a hydrolysable spacer and not as a chiral substituent of a water soluble polymer that interacts noncovalently with another polymer having a lactide substituent of opposite chirality. Thus, WO 98/00170 fails to describe a gel using two types of polymers, each having a chiral substituent that is complementary to the other. Furthermore, unlike the invention as claimed, the hydrogels in WO 98/00170 are prepared by free radical polymerization of a crosslinkable group such as methacrylate, acrylate, vinyl ethers and vinyl esters, resulting in the formation of covalent bonds.

10. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true. Further that these statements were made with the knowledge that willfully false statements, and the like, so made are punishable by fine or imprisonment or both under Section 1001 of Title 18

of the United States Code, and that such willfully false statements may jeopardize the validity of the application of any patent issued thereon.

Date: Jan 20th 2005



Wilhelmus Everhardus Hennink

Short Curriculum vitae Wim E. Hennink

Professor Wim Hennink studied molecular sciences at the Agricultural University of Wageningen, The Netherlands. He graduated in 1981. He obtained his Ph.D. in 1985 at the Twente University of Technology. From April 1985 until November 1989 he worked as a research scientist at the Plastic and Rubber Institute TNO in Delft, the Netherlands. From December 1989 until July 1992 he worked as a development engineer within the R&D Department of Fasson in Leiden, the Netherlands. In July 1992 he took up his present position. From 1996 on he is Head of the Department of Pharmaceutics, Utrecht Institute of Pharmaceutical Sciences, Utrecht University. From from 1997 until 2005 he was European Editor of the Journal of Controlled Release. His main research interests are in the field of polymeric drug delivery systems.

Appendix: list of publication

List of publications, Wim Hennink, January 2005.

1. Van Dedem G, Van Houdenhoven F, and Hennink WE. The USP heparin assay fact and artefact. In: Chemistry and Biology of Heparin (ed. R.L. Lundblad, W.V. Brown, K.G. Mann and H.R. Roberts), Elsevier North Holland Inc., 19-28, 1981.
2. Tramper J, Hennink WE, and Van der Plas HC. Oxidation of 7-alkylpteridin-4-ones to 7-alkyllumanizines. J. Appl. Biochem. 4, 263-270, 1982.
3. Hennink WE, Feijen J, Ebert CD, and Kim SW. Covalently bound conjugates of albumin and heparin: synthesis, fractionation and characterization. Thromb. Res. 29, 1-13, 1983.
4. Feijen J, and Hennink WE. Conjugates of anticoagulant and protein. Eur. Pat. Appl. EP 81853, 1983.
5. Hennink WE, Dost L, Feijen J, and Kim SW. Interaction of albumin-heparin conjugate preadsorbed surfaces with blood. Transactions American Society For Artificial Internal Organs 29, 200-205, 1983.
6. Hennink WE, Ebert CD, Kim SW, Breemhaar W, Bantjes A, and Feijen J. Interaction of antithrombin III with preadsorbed albumin-heparin conjugate. Biomaterials 5, 264-268, 1984.
7. Hennink WE, Kim SW, and Feijen J. Inhibition of surface induced coagulation by preadsorption of albumin-heparin conjugates. J. Biomed. Mater. Res. 18, 911-926, 1984.
8. Hennink WE, Dost L, Kim SW, Van Aken WG, and Feijen J. Blood compatibility of abumin-hparin conjugate treated surfaces. Biomaterials in Artificial Organs (ed. Paul, J.P., Gaylor, J.D.S., Courtney, J.M. and Gilchrist, T.), 203-213, 1983.
9. Hennink WE, Klerrx JPAM, Van Dijk H, and Feijen J. Complement inhibitory and anticoagulant activities of fractionated heparins. Thromb. Res. 36, 281-292, 1984.
10. Hennink WE. Albumin-heparin conjugate as a coating for biomaterials. Thesis University of Twente, Enschede, The Netherlands, 1985.
11. Hennink WE, Knipmeijer AJK, Engbers GHM, Dost L, and Feijen J. Is the use of low molecular weight heparin in blood compatible surface coatings advantageous over that of nonfractionated heparin? Proceedings of the Vth International Conference: Polymers in Medicine and Surgery. Page 7/1-7/11, 1986.
12. Engbers GHM, Dost L, Hennink WE, Aarts PAMM, Sixma JJ, and Feijen J. Platelet adhesion studies onto albumin-heparin conjugate coated catheters. Life Support Syst. 4, 416-418, 1986.
13. Hennink WE, Van den Berg JWA, and Feijen J. Standarization of heparins by means of high performance liquid chromatography equipped with a low angle laser light scattering detector. Thromb. Res. 45, 463-475, 1987.
14. Engbers GHM, Dost L, Hennink WE, Aarts PAMM, Sixma JJ, and Feijen J. An in vitro study of the adhesion of blood platelets onto vascular catheters. Part I. J. Biomed. Mater. Res. 21, 613-627, 1987.
15. Hennink WE. UV-polymerisatie: principes en mogelijkheden. Verfkroniek 61, 260-263, 1988.
16. Huizer L, and Hennink WE. Moisture-permeable polymer network for coating/impregnating. Eur. Pat. Appl. EP 88-201363, 1989.
17. Smit CN, Hennink WE, De Ruiter B, Lulken AH, Marsman MPW, and Bouwma J. Radiation cured halogen free flame-retardant coating. RadTech 90, 148-153, 1990.
18. Deurloo MJM, Bohlken S, Kop W, Lerk CF, Hennink WE, Bartelink H, and Begg AC. Intratumoural administration of cisplatin in slow-release devices. Cancer Chemother. Pharmacol. 27, 135-140, 1990.
19. Hennink WE, and Wilting Th. Cationically hardenable coating compositions, and their application as release coatings. Neth. Pat. Appl., NL 9100226, 1992.
20. Borchert JCH, Van Soest MJ, and Hennink WE. Controlled release of proteins from hydrogels. Proc. Int. Symp. Contr. Rel. Bioact. Mater. 21, 306-307, 1994.
21. Wolthuis WNE, Van Hooff BJM, and Hennink WE. Synthesis and characterization of poly(ethylene glycol) poly-L-lactide block copolymers. Proc. 3rd Eur. Symp. on Controlled Drug Delivery, 271-276, 1994.
22. De Smedt SC, Lauwers A, Demeester J, Van Steenbergen MJ, Hennink WE, and Roefs SPFM. Characterization of the network structure of dextran glycidyl methacrylate hydrogels by studying the rheological and swelling behavior. Macromolecules 28, 5082-5088, 1995.
23. Van Dijk-Wolthuis WNE, Franssen O, Talsma H, Van Steenbergen MJ, Kettenes-van den Bosch JJ, and Hennink WE. Synthesis, characterization and polymerization of

glycidyl methacrylate derivatized dextran. Macromolecules 28, 6317-6322, 1995.
24. Hennink WE, Franssen O, Overbeek AY, Van Steenbergen MJ, and Talsma H. Enzymatic Degradation of dextran hydrogels. Proc. Int. Symp. Contr. Rel. Bioact. Mater. 22, 23-24, 1995.
25. Hennink WE, Talsma H, Borchert JCH, De Smedt SC, and Demeester J. Controlled release of proteins from dextran hydrogels. J. Control. Release 39, 47-55, 1996.
26. Cherng JY, Van de Wetering P, Talsma H, Crommelin DJA, and Hennink WE. Effect of size and serum proteins on transfection efficiency of poly((2-dimethylamino)ethylmethacrylate)-plasmid nanoparticles. Pharm. Res. 13, 1038-1042, 1996.
27. Van Dijk-Wolthuis WNE, Tsang SKY, and Hennink WE. Biodegradable dextran hydrogels. Proc. Int. Symp. Contr. Rel. Bioact. Mater. 23, 755-756, 1996.
28. Hennink WE, Feijen J, and Crommelin DJA. Gecontroleerde en gerichte afgifte geneesmiddelen; 'slimme' verpakking wijst de weg en stuurt dosering. Chemisch Magazine, oktober 1996, 413-415.
29. Franssen O, Vos OP, and Hennink WE. Delayed release of a model protein from enzymatically degrading dextran hydrogels. J. Control. Release 44, 237-245, 1997.
30. Hennink WE, Franssen O, Van Dijk-Wolthuis WNE, and Talsma H. Dextran hydrogels for the controlled release of proteins. J. Control. Release 48, 107-114, 1997.
31. Van de Wetering P, Cherng JY, Talsma H, and Hennink WE. Relation between the transfection efficiency and cytotoxicity of poly((2-dimethylamino)- ethyl methacrylate)-plasmid complexes. J. Control. Release 49, 59-71, 1997.
32. Van der Voort Maarschalk K, Zuurman K, Van Steenbergen MJ, Hennink WE, Vromans H, Bolhuis GK, and Lerk CF. Effect of compaction temperature on consolidation of amorphous copolymers with different glass transition temperatures. Pharm. Res. 14, 415-419, 1997.
33. Van Dijk-Wolthuis WNE, Van Steenbergen MJ, Underberg WJM, and Hennink WE. Degradation kinetics of methacrylated dextrans in aqueous solution. J. Pharm. Sci. 86, 413-417, 1997.
34. Schlicher EJAM, Postma N, Zuidema J, Talsma H, and Hennink WE. Preparation and characterization of poly(d,l-lactic-co-glycolic acid) microspheres containing desferrioxamine. Int. J. Pharm.153, 235-245, 1997.
35. Van Dijk-Wolthuis WNE, Kettenes-van den Bosch JJ, Van der Kerk-van Hoof A, and Hennink WE. Reaction of dextran with glycidyl methacrylate: an unexpected transesterification. Macromolecules 30, 3411-3413, 1997.
36. Van Dijk-Wolthuis WNE, Tsang SKY, Kettenes-van den Bosch JJ, and Hennink WE. A new class of polymerizable dextrans with hydrolyzable groups: hydroxyethyl methacrylated dextran with and without oligolactate spacer. Polymer 38, 6235-6242, 1997.
37. Van Dijk-Wolthuis WNE, Van de Water L, Van de Wetering P, Van Steenbergen MJ, Kettenes-van den Bosch JJ, Schuyl PJW, and Hennink WE. Synthesis and characterization of poly-L-lysine with controlled low molecular weight. Macromol. Chem. Phys. 198, 3893-3907, 1997.
38. Van Dijk-Wolthuis WNE, Tsang SKY, Van Steenbergen MJ, Hoogeboom C, and Hennink WE. Degradation and release behaviour of dextran based hydrogels. Macromolecules 30, 4639-4645, 1997.
39. De Smedt SC, Meyvis TKL, Demeester J, Van Oostveldt P, Blonk JCG, and Hennink WE. The diffusion of macromolecules in dextran glycidyl methacrylate solutions and gels as studied by confocal laser microscopy. Macromolecules 30, 4863-4870 1997.
40. Hennink WE, Feijen J, and Crommelin DJA. 'Routeplanner' helpt geneesmiddel zieke plaats te vinden. Liposomen die cytostatica bevatten. Conceptuur 11, 6-8, 1997.
41. Talsma H, Cherng JY, Lehrmann H, Malgorzata K, Ogris M, Hennink WE, Cotton M, and Wagner E. Stabilization of gene delivery systems by freeze-drying. Int. J. Pharm. 157, 233-238, 1997.
42. Franssen O, Van Ooijen RD, De Boer D, Maes RAA, Herron JN, and Hennink WE. Enzymatic degradation of methacrylated dextrans. Macromolecules 30, 7408-7413, 1997.
43. Cherng JY, Van de Wetering P, Talsma H, Crommelin DJA, and Hennink WE. Freeze-drying of poly((2-dimethylamino)ethyl methacrylate)-based gene delivery systems. Pharm. Res. 14, 1837-1840, 1997.
44. Hennink WE, and Van de Wetering Petra. Cationic polyacrylates and poly(alkyl) acrylates and acrylamides for use as carriers of nucleic acid in the transformation of

animal cells. PCT Int. Appl., WO 9715680, 1997.
45. Hennink WE, and Talsma H. Pharmaceutical applications of gelatin. Industrial Proteins 4, 5-6, 1997.
46. Hennink WE, and Bout A. A transfection system using poly(organo)phosphazenes complexes with nucleic acids. PCT Int. Appl. WO 9707226, 1997.
47. Van de Wetering P, Cherng JY, Gutierez-Camacho P, Talsma H, and Hennink WE. Relation between the transfection efficiency and cytotoxicity of poly(DMAEMA)/plasmid complexes. Proc. Int. Symp. Contr. Rel. Bioact. Mater. 24, 655-656, 1997.
48. Van de Wetering P, Cherng JY, Talsma H, Crommelin DJA, and Hennink WE. 2-(Dimethylaminoethyl) methacrylate based polymers and copolymers as gene delivery agents. J. Control. Release 53, 145-155, 1998.
49. Goedemoed JH, and Hennink WE. Polyetherester copolymers as drug delivery matrixes. PCT Int. Appl. WO 9806439, 1998.
50. Stenekes RJH, Franssen O, van Bommel EMG, Crommelin DJA, and Hennink WE. The preparation of dextran microspheres in an all-aqueous system: effect of the formulation parameters on particle characteristics. Pharm. Res. 15, 557-561, 1998.
51. Hennink WE, and Van Dijk-Wolthuis WNE. Hydrolyzable hydrogels comprising two interpenetrating polymer networks for controlled release. PCT Int. Appl. WO 9800170, 1998.
52. Franssen O, and Hennink WE. A Novel preparation method for polymeric microparticles without the use of organic solvents. Int. J. Pharm. 168, 1-7, 1998.
53. Franssen O, and Hennink WE. Preparation of dextran microspheres by in all-aqueous system. Proc. Int. Symp. Contr. Rel. Bioact. Mater. 25, 232-233, 1998.
54. Meyvis TKL, van Oostveldt P, Hennink WE, and Demeester J. Correlation between rheological characteristics of enzymatically degrading dextran glycidyl methacrylate gels and the mobility of macromolecules inside these gels. In: Chemical and Physical Networks, Formation and control of properties, K te Nijenhuis and W.J. Mijs, ed., John Wiley and sons, 1998, 527-535.
55. De Jong SJ, Van Dijk-Wolthuis WNE, Kettenes-van den Bosch JJ, Schuyl PJW, and Hennink WE. Monodisperse enantiomeric lactic acid oligomers: preparation, characterization and stereocomplex formation. Macromolecules 31, 6397-6402, 1998.
56. Van de Wetering P, Zuidam NJ, Van Steenbergen MJ, Van der Houwen OAGJ, Underberg WJM, and Hennink WE. A mechanistic study of the hydrolytic stability of poly(2-(dimethyl)aminoethyl methacrylate). Macromolecules 31, 8063-8068, 1998.
57. Hennink WE, and Franssen O. Process for the preparation of a controlled release system, WO9822093, 1998.
58. Van de Weert M, Van 't Hof R, Hennink WE, and Crommelin DJA. Stability of pharmaceutical proteins in particulate carriers. In: Peptide and Protein Drug Delivery (Frokjaer S, Christrup L and Krogsgaard-Larsen P, Eds.), Munksgaard, Copenhagen, 359-375, 1998.
59. Talsma H, Cherng JY, Van de Wetering P, Crommelin DJA, and Hennink WE. Poly(2-(dimethyl)aminoethyl methacrylate) nanoparticles for gene transfection. In: Future Strategies for Drug Delivery with Particulate Systems, J.E. Diederichs and R.H. Muller, medpharm. GmbH Scientific publishers, Stuttgart, Germany, 177-183, 1998.
60. Crommelin DJA, Hennink WE, and Storm G. Strategies for site specific, time and rate controlled delivery of drugs, genes and antigenes. In: In: Future Strategies for Drug Delivery with Particulate Systems, J.E. Diederichs and R.H. Muller, medpharm. GmbH Scientific publishers, Stuttgart, Germany, 127-132, 1998.
61. Wink T, De Beer J, Hennink WE, Bult A, and Van Bennekom WP. Interaction between plasmid DNA and cationic polymers studied by surface plasmon resonance spectrometry. Anal. Chem. 71, 801-805, 1999.
62. Franssen O, Van Rooijen RD, De Boer D, Maes RAA, and Hennink WE. Enzymatic degradation of crosslinked dextrans. Macromolecules 32, 2896-2902, 1999.
63. Cherng JY, Talsma H, Verrijk R, Crommelin DJA, and Hennink WE. The effect of formulation parameters on the size of poly((2-dimethylamino)ethyl methacrylate)-plasmid complexes. Eur. J. Pharm. Biopharm. 47, 215-224, 1999.
64. Franssen O, Stenekes RJH, and Hennink WE. Controlled release of a model protein from enzymatically-degrading dextran microspheres J. Control. Release 59, 219-228, 1999.
65. Franssen O, Vandervennet L, Roders P, and Hennink WE. Degradable dextran hydrogels: controlled release of a model protein from cylinders and microspheres. J.

Control. Release 60, 211-221, 1999.
66. Van Es RJJ, Franssen O, Dullens HFJ, Bernsen MR, Bosman F, Hennink WE, and Slootweg PJ. The VX2 carcinoma in the rabbit auricle as an experimental model for the intra-arterial embolization of head and neck squamous cell carcinoma with dextran microspheres. Lab. Anim. 33, 175-184, 1999.
67. Hinrichs WLJ, Schuurmans-Nieuwenbroek NME, Van de Wetering P, and Hennink WE. Thermosensitive polymers as carriers for DNA delivery. J. Control. Release 60, 249-259, 1999.
68. Cheng JY, Schuurmans-Nieuwenbroek NME, Jiskoot W, Talsma H, Zuidam NJ, Hennink WE, and Crommelin DJA. Effect of DNA topology on the transfection efficiency of poly(2-(dimethylamino)ethyl methacrylate)-plasmid complexes. J. Control. Release 60, 343-353, 1999.
69. Van de Wetering P, Moret EE, Schuurmans-Nieuwenbroek NME, Van Steenbergen MJ, and Hennink WE. Structure-activity relationships of water-soluble cationic methacrylate/methacrylamide polymers for nonviral gene delivery. Bioconjugate Chem. 10, 687-692, 1999.
70. Meyvis TKL, De Smedt SC, Demeester J, and Hennink WE. Rheological monitoring of long-term degrading polymer hydrogels. J. Rheol. 43, 933-950 1999.
71. Stenekes RJH, and Hennink WE. Equilibrium water content of microspheres based on crosslinked dextran. Int. J. Pharm. 189, 62-65, 1999.
72. Arigita C, Zuidam NJ, Crommelin DJA, and Hennink WE. Association and dissociation characteristics of polymer/DNA complexes used for gene delivery. Pharm. Res. 16, 1534-1541, 1999.
73. Van Dijk-Wolthuis WNE, Van de Wetering P, Hinrichs WLJ, Hofmeyer LJF, Lislcamp RMJ, Crommelin DJA, and Hennink WE. A versatile method for the conjugation of proteins and peptides to poly(2-(dimethylamino)ethyl methacrylate. Bioconjugate Chem. 10, 589-597, 1999.
74. Cadée JA, De Kerf M, De Groot CJ, Den Otter W, and Hennink WE. Synthesis and characterization of 2-(methacryloyloxy)ethyl-(di)-L-lactate and their application in dextran-based hydrogels. Polymer 40, 6877-6881, 1999.
75. Cheng JY, Talsma H, Crommelin DJA, and Hennink WE. Long term stability of poly((2-dimethylamino)ethyl methacrylate)-based gene delivery systems. Pharm. Res. 16, 1417-1423, 1999.
76. Den Otter W, Balemans L, Batterman JJ, Bernsen MR, Cadée JA, Dobrowolski Z, Everse LA, Fiszer-Maliszewska L, Gavhumende R, De Groot JW, De Groot K, Hennink WE, Graham Hill FW, Jurgenliemp-Schulz I, Klein WR, Koten JW, Maas RA, Steerenberg P, Stewart R, and Zembala M. Local low-dose IL-2 therapy, Hepato-Gastroeneterology 46, 1280-1286, 1999.
77. Van de Wetering P, Schuurmans-Nieuwenbroek NME, Hennink WE, and Storm G. Comparative transfection studies of human ovarian carcinoma cells in vitro, ex vivo and in vivo with poly(2-(dimethylamino)ethyl methacrylate)-based polyplexes. J. Gene Med. 1, 156-165, 1999.
78. Stenekes RJH, Franssen O, Van Bommel EM, Crommelin DJA, and Hennink WE. The use of aqueous PEG/dextran phase separation for the preparation of dextran microspheres. Int. J. Pharm. 183, 29-32, 1999.
79. Cheng JY, Van de Wetering P, Talsma H, Crommelin DJA, and Hennink WE. Stabilization of polymer-based gene delivery systems. Int. J Pharm. 183, 25-28 1999.
80. Den Otter W, Cadée JA, Gavhumende R, De Groot CJ, Hennink WE, and Stewart R. Effective cancer therapy with a single injection of interleukin-2 at the site of the tumour. Cancer Immunol. Immunother. 48, 419-420, 1999.
81. De Jong SJ, Van Dijk-Wolthuis WNE, Kettenes-van den Bosch JJ, and Hennink WE. Stereocomplex formation in enantiomeric lactic acid oligomers. Proc. Int. Symp. Contr. Rel. Bioact. Mater. 26, 691-692, 1999.
82. Fonseca MJ, Storm G, Hennink WE, Gerritsen WR, and Halsma HJ. Cationic polymeric gene delivery of Π-glucuronidase for doxorubicin prodrug therapy. J. Gene Med. 1, 404-417, 1999.
83. Neradovic D, Hinrichs WLJ, Kettenes-van den Bosch JJ, and Hennink WE. Poly(N-isoprpoylacrylamide) with hydrolyzable lactic acid side groups: a new type of thermosensitive polymer. Macromol. Rapid Commun. 20, 577-581, 1999.
84. Van de Wetering P, Van Steenbergen MJ, Crommelin DJA, and Hennink WE. Copolymers of 2-dimethylaminoethyl methacrylate with ethoxytriethylene glycol methacrylate or N-vinyl-pyrrolidone as gene transfer agents. J. Control. Release 64, 193-203, 2000.

85. Stenekes RJH, and Hennink WE. Polymerization kinetics of dextran-bound methacrylate in an aqueous two phase system. Polymer 41, 5563-5569, 2000.
86. Cadée JA, Van Luyn MJA, Brouwer LA, Plantinga JA, Van Wachem PB, De Groot CJ, Den Otter W, and Hennink WE. In vivo biocompatibility of dextran-based hydrogels. J. Biomed. Mater. Res. 50, 397-404, 2000.
87. De Smedt SC, Demeester J, and Hennink WE. Cationic polymer based gene delivery systems. Pharm. Res. 17, 113-126, 2000.
88. Zuidam NJ, Posthuma G, De Vries BT, Crommelin DJA, Hennink WE, and Storm G. Effects of physicochemical characteristics of poly(2-(dimethyl-amino)ethyl methacrylate)-based polyplexes on cellular association and internalization. J. Drug Target. 8, 51-66, 2000.
89. De Jong SJ, De Smedt SC, Wahls MWC, Demeester J, Kettenes-van den Bosch JJ, and Hennink WE. Novel self-assembled hydrogels by stereocomplex formation in aqueous solution of enantiomeric lactic acid oligomers grafted to dextran. Macromolecules 33, 3680-3686, 2000.
90. Meyvis TKL, De Smedt SC, Demeester J, and Hennink WE. Influence of the degradation mechanism of hydrogels on their elastic and swelling properties during degradation. Macromolecules 33, 4717-4725, 2000.
91. Van de Weert M, Van 't Hof R, Van der Weerd J, Heeren RM, Posthuma G, Hennink WE, and Crommelin DJA. Lysozyme distribution and conformation in a biodegradable polymer matrix as determined by FTIR techniques. J. Control. Release 68, 31-40, 2000.
92. Stenekes RJH, Loebis AE, Fernandes CM, Crommelin DJA, and Hennink WE. Controlled release of liposomes from biodegradable dextran microspheres: a novel delivery concept. Pharm. Res. 17, 690-695, 2000.
93. Hennink WE, Cadée JA, De Jong, SJ, Franssen O, Stenekes RJH, Van Nostrum RN, Talsma H, and Van Dijk-Wolthuis WNE. Biodegradable hydrogels for the controlled release of pharmaceutical applications. In: Biomaterials and drug delivery toward new millennium (Park KD, Kwon IC, Yui N, Jeong SY, Park K. Eds.) Han Rim Won Publishing, 131-147, 2000.
94. Van de Weert M, Hoechstetter J, Hennink WE, and Crommelin DJA. The effect of a water/organic interface on the structural stability of lysozyme. J. Control. Release 68, 351-359, 2000.
95. Storm G, Van de Wetering P, Zuidam, NJ, Cherng JY, Talsma H, Crommelin DJA, and Hennink WE. Methacrylate-based polyplexes as transfection agent. NATO Sci. Ser., Ser. A 323 (Targeting of Drugs: Strategies for Gene Constructs and Delivery), 192-199, 2000.
96. Cadée JA, Van Luyn MJA, Brouwer LA, Van Wachem PB, De Groot CJ, Den Otter W, and Hennink WE. In vivo biocompatibility and interleukin-2 release behaviour of dextran-based hydrogels. Proc. Int. Symp. Controlled Release Bioact. Mater., 1056-1057, 2000.
97. Van de Weert M, Hennink WE, and Crommelin DJA. Lysozyme structure and stability in poly(lactic-co-glycolic acid) microspheres. Proc. Int. Symp. Controlled Release Bioact. Mater., 962-963, 2000.
98. Talsma H, Cherng JY, Crommelin DJA, and Hennink WE. Long term stability of gene delivery systems based on poly((2-dimethylamino)ethyl methacrylate)/plasmid complexes. Proc. Int. Symp. Controlled Release Bioact. Mater., 826-827, 2000.
99. De Jong SJ, De Smedt SC, Kettenes-van den Bosch JJ, Hennink WE. Hydrogels formed by physical interaction of enantiomeric lactic acid oligomers grafted to dextran. Proc. Int. Symp. Controlled Release Bioact. Mater., 680-681, 2000.
100. Neradovic D, Hinrichs WLJ, Kettenes-van den Bosch JJ, Van Nostrum CF, and Hennink WE. A new type of thermosensitive polymer: Poly(N-isopropylacrylamide) with hydrolyzable lactic acid ester side groups. Proc. Int. Symp. Controlled Release Bioact. Mater., 612-613, 2000.
101. Stenekes RJH, Loebis AE, Fernandes CM, Crommelin DJA, and Hennink WE. A novel delivery system based on liposomes entrapped in biodegradable dextran microspheres. Proc. Int. Symp. Controlled Release Bioact. Mater., 281-282, 2000.
102. Srenekes RJH, de Smedt SC, Demeester J, Sun G, Zhang Z, and Hennink WE. Pore sizes in hydrated dextran microspheres. Biomacromolecules 1, 696-703, 2000.
103. Hennink WE, and Crommelin DJA. Stabilisation and storage of nucleic acids for transformation when not incorporated into viruses. Eur. Pat. Appl., EP 1022340, 2000.
104. Van de Weert M, Hennink WE, and Jiskoot W. Protein instability in poly(lactic-co-

glycolic acid) microparticles. Pharm. Res. 17, 1159-1167, 2000.
105. Hennink WE., Van Nostrum CF, and De Jong SJ. Stereocomplex hydrogels. PCT Int. Appl. WO 0048576 A1, 2000.
106. Bos GW, Trullas-Jimeno A, Jiskoot W, Crommelin DJA, and Hennink WE. Sterilization of poly(dimethylamino)ethyl methacrylate-based gene transfer complexes. Int. J. Pharm. 211, 79-88, 2000.
107. De Jong SJ, Ruiz-Arias E, Rijkers DTS, van Nostrum CF, Kettenes-van den Bosch JJ, and Hennink WE. New insights into the hydrolytic degradation of poly(lactic acid): Participation of the alcohol terminus. Polymer 42, 2795-2802, 2001.
108. Sicendam R, Van Steenbergen MJ, Hennink WE, Frijlink HW, and Lerk CF. Effect of molecular weight and glass transition on relaxation and release behaviour of poly(dl-lactic acid) tablets. J. Control. Release 70, 71-82, 2001.
109. Hennink WE, Hinrichs WLJ, and Mulders-Neradovic D. Temperature sensitive polymers. Eur. Pat. Appl. EP 1072617, 2001.
110. Hennink WE, Cherng JY, Jiskoot W, Talsma H, and Crommelin DJA. Pharmaceutical aspects of polymer-based non-viral gene delivery systems: experience with p(DMAEMA)-pCMV-lacZ polyplexes. S.T.P. Pharma Sciences 11, 11-19, 2001.
111. Stenekes RJH, Loebis AE, Fernandes CM, Crommelin DJA, and Hennink WE. Degradable dextran microspheres for the controlled release of liposomes. Int. J. Pharm. 214, 17-20, 2001.
112. Verbaan FJ, Oussoren O, Van Dam IM, Takakura Y, Hashida M, Crommelin DJA, Hennink WE, and Storm G. The fate of poly(2-dimethyl amino ethyl)methacrylate-based polyplexes after intravenous administration. Int. J. Pharm. 214, 99-101, 2001.
113. De Jong SJ, Van Eerdenbrugh B, Van Nostrum CF, Kettenes-van den Bosch JJ, and Hennink WE. Physically crosslinked dextran hydrogels by stereocomplex formation of lactic acid oligomers: degradation and protein release behavior. J. Control. Release 71, 261-275, 2001.
114. De Jong SJ, De Smedt SC, Demeester J, Van Nostrum CF, Kettenes-van den Bosch JJ, and Hennink W.E. Biodegradable hydrogels based on stereocomplex formation between lactic acid oligomers grafted to dextran. J. Control. Release 72, 47-56, 2001.
115. Yilmaz G, Jongboom ROJ, Feil H, and Hennink WE. Encapsulation of sunflower oil in starch matrices via extrusion: effect of the interfacial properties and processing conditions on the formation of dispersed phase morphologies. Carbohydr. Polym. 45, 403-410, 2001.
116. De Groot CJ, Van Luyn MJA, Van Dijk-Wolthuis WNE, Cadée JA, Plantinga JA, Den Otter W, and Hennink WE. In vitro biocompatibility of biodegradable dextran-based hydrogels tested with human fibroblasts. Biomaterials 22, 1197-1203, 2001.
117. Stenekes RJH, Talsma H, and Hennink WE. Formation of dextran hydrogels by crystallization. Biomaterials 22, 1891-1898, 2001.
118. Nijsen F, Rook D, Brandt C, Meijer R, Dullens H, Zonnenberg B, De Klerk J, Van Rijk P, Hennink WE, Van het Schip AD. Targeting of liver tumour in rats by selective delivery of holmium-166 loaded microspheres: a biodistribution study. Eur. J. Nucl. Med. 28, 743-749, 2001.
119. Cadée JA, Brouwer LA, Den Otter W, Hennink WE, and Van Luyn MJA. A comparative biocompatibility study of microspheres based on crosslinked dextran or poly(lactic-co-glycolic)acid after subcutaneous injection in rats. J. Biomed. Mater. Res. 56, 600-609, 2001.
120. Mastrobattista E, Kapel RHG, Eggenhuisen MH, Roholl PJM, Crommelin DJA, Hennink WE, and Storm G. Lipid-coated polyplexes for targeted gene delivery to ovarian carcinoma cells. Cancer Gene Ther. 8, 405-413, 2001.
121. Nijssen JWF, Van Steenbergen MJ, Kooijman H, Talsma H, Kroon-Batenburg LMJ, Van de Weert M, Van Rijk PP, De Witte A, Van het Schip AD, and Hennink WE. Characterization of poly(L-lactic acid) microspheres loaded with holmium acetylacetonate. Biomaterials 22, 3073-3081, 2001.
122. Hennink WE, Cadée JA, De Jong SJ, Franssen RJH, Talsma H, and Van Dijk-Wolthuis WNE. Molecular design of degradable dextran hydrogels for the controlled release of proteins. In: Biomedical Polymers and Polymer Therapeutics. Edited by Emo Chiellini, Junzo Sunamoto, Claudio Migliaresi, Raphael M. Ottenbrite and Daniel Cohn. Kluwer Academic/Plenum Publishers, New York, 3-18, 2001.
123. Hennink WE, Van de Wetering P, Storm G, and Crommelin DJA. Gene delivery with water-soluble cationic methacrylate/methacrylamide polymers. In: Proceedings of the 6th International Symposium on Adjuvants for Agrochemicals. Edited by Hans de

Ruiter. 595-607, 2001.
124. Cadée JA, Van Steenbergen MJ, Versluis C, Heck AJR, Underberg WJM, Den Otter W, Jiskoot W, and Hennink WE. Oxidation of interleukin-2 by potassium peroxodisulfate. Pharm. Res. 18, 1461-1467, 2001.
125. Hennink WE, and Franssen O. Preparation of microspheres containing colloidal systems. PCT Int. Appl., WO 0160339 A2 20010823, 2001.
126. Bos GW, Crommelin DJA, and Hennink WE. Water-soluble cationic methacrylate polymers for nonviral gene delivery. Methods Mol. Med., 65, 35-60, 2001.
127. Neradovic D, Van Nostrum CF, and Hennink WE. Thermoresponsive polymeric micelles with controlled instability based on hydrolytically sensitive N-isopropylacrylamide copolymers. Macromolecules 34, 7589-7591, 2001.
128. Van de Weert M, Haris PI, Hennink WE, and Crommelin DJA. Fourier transform infrared spectrometric analysis of protein conformation: effect of the sampling method and stress factors. Anal. Biochem. 297, 160-169, 2001.
129. Bos GW, Verrijk R, Franssen O, Bezemer JM, Hennink WE, and Crommelin DJA. Hydrogels for the controlled release of pharmaceutical proteins. Pharmaceutical Technology 25, 110-120, 2001.
130. Meyvis TKL, De Smedt SC, Stubbe B, Hennink WE, and Demeester J. On the release of proteins from degrading dextran methacrylate hydrogels and the correlation with rheologic properties of the hydrogels. Pharm. Res. 18, 1593-1599, 2001.
131. Bos GW, Verrijk R, Franssen O, Bezemer JM, Hennink WE, and Crommelin DJA Hydrogels for the controlled release of pharmaceutical proteins. Biopharm Europe 13, 64-74, 2001.
132. Van Es RJJ, Nijsen FW, Dullens HFJ, Kicken M, Van der Bilt A, Hennink WE, Kolle R, and Slootweg PJ. Tumour embolization of the Vx2 rabbit head and neck cancer model with dextran hydrogel and holmium-poly(L-lactic acid) microspheres: a radionuclide and histological pilot study. J. Cranio-MaxilloFac. Surg. 29, 289-297, 2001.
133. Crommelin DJA, Hennink WE and Storm G. Drug targeting systems: fundamental and applications to parenteral drug delivery. In: Drug Delivery and Applications to Parenteral Drug Delivery. Edited by: A.M. Hillery, A.W. Lloyd and J. Swarbick. Page 117-144, 2001.
134. Storm G, Mastrobattista E, Verbaan FJ, Crommelin DJA, and Hennink WE. Design of methacrylate-based polyplexes for tumor targeting. Gene Ther. Mol. Biol. 6, 143-148, 2001.
135. Hennink WE, and Van Nostrum CF. Novel crosslinking methods to design hydrogels. Adv. Drug Del. Rev. 54, 13-36, 2002.
136. Cadée JA, De Groot CJ, Jiskoot W, Den Otter W, and Hennink WE. Release of recombinant human interleukin-2 from dextran-based hydrogels. J. Control. Release 78, 1-13, 2002.
137. Nijsen JWF, Van het Schip AD, Hennink WE, Rook DW, Van Rijk PP, and De Klerk JMH. Advances in nuclear oncology: Microspheres for internal radionuclide therapy of liver tumours. Curr. Med. Chem. 9, 73-82, 2002.
138. De Groot CJ, Cadée JA, Koten JW, Hennink WE, and Den Otter W. Therapeutic efficacy of IL-2-loaded hydrogels in a mouse tumor model. Int. J. Cancer 98, 134-140, 2002.
139. Nijsen JWF, Van het Schip AD, Van Steenbergen MJ, Zielhuis SW, Kroon-Batenburg LMJ, Van de Weert M, Van Rijk PP, and Hennink WE. Influence of neutron irradiation on holmium acetylacetonate loaded poly(l-lactic acid) microspheres. Biomaterials 23, 1831-1839, 2002.
140. Stubbe BG, Braeckmans K, Horkay F, Hennink WE, De Smedt SC, and Demeester J. Swelling pressure observations on degrading dex-HEMA hydrogels. Macromolecules 35, 2501-2505, 2002.
141. Yilmaz G, Oengen G, Jonghoom ROJ, Feil H, Van Dijk C, and Hennink WE. Modulated release of a volatile compound from starch matrixes via enzymatically controlled degradation. Biomacromolecules 3, 305-311, 2002.
142. De Jong SJ, Van Nostrum CF, Kroon-Batenburg LMJ, Kettenes-van den Bosch JJ, and Hennink WE. Oligolactate-grafted dextran hydrogels: detection of stereocomplex crosslinks by X-ray diffraction. J. Appl. Polym. Sci. 86, 289-293, 2002.
143. Meyvis TKL, Stubbe BG, Van Steenbergen MJ, Hennink WE, De Smedt SC, and Demeester J. A comparison between the use of dynamic mechanical analysis and oscillatory shear rheometry for the characterisation of hydrogels. Int. J. Pharm. 244, 163-168, 2002.

144. Van de Weert M, Van Steenbergen MJ, Cleland JL, Heller J, Hennink WE, and Crommelin DJA. Semisolid, self-catalyzed poly(ortho ester)s as controlled-release systems: protein release and protein stability issues. J. Pharm. Sci. 91, 1065-1074, 2002.
145. Van de Weert M, van Dijkhuizen-Radersma R, Bezemer JM, Hennink WE, and Crommelin DJA. Reversible aggregation of lysozyme in a biodegradable amphiphilic multiblock copolymer. Eur. J. Pharm. Biopharm. 54, 89-93, 2002.
146. Metselaar JM, Hennink WE, De Vringer T, De Boer LWT, Oussoren C, Storm G, and Bruin P. Lipid-polymer conjugate compositions for colloidal carriers. PCT Int. Appl. A1 20021212, 2002.
147. Arigita C, Kersten GFA, Hazendonk T, Hennink, WE, Crommelin DJA, Jiskoot W. Restored functional immunogenicity of purified meningococcal PorA by incorporation into liposomes. Vaccine 21, 950-960, 2003.
148. Crommelin DJA, Storm G, Jiskoot W, Stenekes R, Mastrobattista E, and Hennink WE Nanotechnological approaches for the delivery of macromolecules. J. Control. Release 87, 81-88, 2003
149. Van Steenis JH, Van Maarseveen EM, Verbaan FJ, Verrijk R, Crommelin DJA, Storm G, and Hennink WE. Preparation and characterization of folate-targeted pEG-coated pDMAEMA-based polyplexes. J. Control. Release 87, 167-176, 2003
150. Luten J, Van Steenis JH, Van Someren R, Kemmink J, Schuurmans-Nieuwenbroek NME, Koning GA, Crommelin DJA, Van Nostrum CF, and Hennink WE. Water-soluble biodegradable cationic polyphosphazenes for gene delivery. J. Control. Release 89, 483-497, 2003.
151. Stubbe BG, Horkay F, Amsden B, Hennink WE, De Smedt SC, and Demeester J. Tailoring the swelling pressure of degrading dextran hydroxyethyl methacrylate hydrogels. Biomacromolecules 4, 691-695, 2003.
152. Arigita C, Bevaart L, Everse LA, Koning GA, Hennink WE, Crommelin DJA, Van de Winkel JGJ, Van Vugt MJ, Kersten GFA, and Jiskoot W. Liposomal meningococcal B vaccination: Role of dendritic cell targeting in the development of a protective immune response. Infect. Immun. 71, 5210-5218, 2003.
153. Leemhuis M, Van Steenis JH, Van Uxem MJ, Van Nostrum CF, and Hennink WE. A versatile route to functionalized dilactones as monomers for the synthesis of poly(alpha-hydroxy) acids. Eur. J. Org. Chem. 17, 3344-3349, 2003.
154. Neradovic D, Van Steenbergen MJ, Vansteelant L, Meijer YJ, Van Nostrum CF, and Hennink WE. Degradation mechanism and kinetics of thermosensitive polyacrylamides containing lactic acid side chains. Macromolecules 36, 7491-7498, 2003.
155. Van Nostrum CF, De Jong SJ, Kettenes-van den Bosch JJ, Hennink WE. Hydrogels by stereocomplex formation and their use as drug delivery matrices. Advances in Controlled Drug Delivery: Science, Technology, and Products. ACS Symposium Series 846, 129-141, 2003.
156. Crommelin DJA, Storm G, Verrijk R, De Leede L, Jiskoot W, and Hennink WE. Shifting paradigms: biopharmaceuticals versus low molecular weight drugs. Int. J. Pharm., 266, 3-16, 2003.
157. Koten JW, Van Luyn MJA, Cadée JA, Bouwer L, Hennink WE, Bijleveld C, and Den Otter W. IL-2 loaded dextran microspheres with attractive histocompatibility properties for local IL-2 cancer therapy. Cytokine 24, 57-66, 2003.
158. Metselaar JM, Bruin P, De Boer LWT, De Vringer T, Snel C, Oussoren C, Wauben MHM, Crommelin DJA, Storm G, and Hennink WE. A novel family of L-amino acid-based biodegradable polymer-lipid conjugates for the development of long-circulating liposomes with effective drug-targeting capacity. Bioconjugate Chem. 14, 1156-1164, 2003.
159. Metz B, Jiskoot W, Hennink WE, Crommelin DJA, and Kersten GFA. Physicochemical and immunochemical techniques predict the quality of diphtheria toxoid vaccines. Vaccine 21, 156-167, 2003.
160. Verbaan F, Van Dam I, Takakura Y, Hashida M, Hennink WE, Storm G, and Oussoren C. Intravenous fate of poly(2-(dimethylamino)ethyl methacrylate)-based polyplexes. Eur. J. Pharm. Sciences 20, 419-427, 2003.
161. Funhoff AM, Van Nostrum CF, Janssen APCA, Fens MHAM, Crommelin DJA, and Hennink WE. Polymer side-chain degradation as a tool to control the destabilization of polyplexes. Pharm. Res. 21, 170-176, 2004.
162. Verbaan FJ, Oussoren C, Snel CJ, Crommelin DJA, Hennink WE, and Storm G. Steric stabilization of poly(2-(dimethylamino)ethyl methacrylate)-based polyplexes

mediates prolonged circulation and tumor targeting in mice. J. Gene Med. 6, 64-75, 2004.
163. Funhoff AM, Van Nostrum CF, Koning GA, Schuurmans-Nieuwenbroek NME, Crommelin DJA, and Hennink WE. Endosomal escape of polymeric gene delivery complexes is not always enhanced by polymers buffering at low pH. Biomacromolecules 5, 32-39, 2004.
164. Neradovic D, Soga O, Van Nostrum CF, and Hennink WE. The effect of the processing and formulation parameters on the size of nanoparticles based on block copolymers of poly(ethylene glycol) and poly(*N*-isopropylacrylamide) with and without hydrolytically sensitive groups. Biomaterials 25, 2409-2418, 2004.
165. Arigita C, Jiskoot W, Westdijk J, Van Ingen C, Hennink WE, Crommelin DJA, and Kersten GFA. Stability of mono- and trivalent meningococcal outer membrane vesicle vaccines. Vaccine 22, 629-642, 2004.
166. Arigita C, Van den Berg J, Wensink K, Van Steenbergen MJ, Hennink WE, Crommelin DJA, Kersten GFA, and Jiskoot W. Immunogenicity of meningococcal PorA formulations encapsulated in biodegradable microspheres. European Journal of Pharmaceutical Sciences 21, 131-141, 2004.
167. Metz B, Kersten GF, Hoogerhout P, Timmermans HA, De Jong A, Meiring H, Ten Hove J, Hennink WE, Crommelin DJA, and Jiskoot W. Identification of formaldehyde-induced modifications in proteins. Reactions with model peptides. J. Biol. Chem. 279, 6235-6243, 2004.
168. Bos GW, Jacobs JJL, Koten JW, Van Tomme S, Veldhuis Th, Van Nostrum CF, Den Otter W, and Hennink WE. In situ crosslinked biodegradable hydrogels loaded with IL-2 are effective tools for local IL-2 delivery. Eur. J. Pharm. Sciences 21, 561-567, 2004.
169. Yilmaz G, Jongboom ROJ, Feil H, Van Dijk C, and Hennink WE. Permeation of volatile compounds through starch films. Biomacromolecules 5, 650-656, 2004.
170. Van Nostrum CF, Veldhuis TFJ, Bos GW, and Hennink WE. Tuning the degradation rate of poly(2-hydroxypropyl methacrylamide)-graft-oligo(lactic acid) stereocomplex hydrogels. Macromolecules 37, 2113-2188, 2004.
171. Hennink WE, Van Steenis JH, and Van Nostrum CF. Fast degradable polymers. In: Reflexive Polymers and Hydrogels. Understanding and designing fast responsive polymeric system. Ed: Yui N, Mrsny RJ, and Park K. CRC Press. Page 401-423, 2004.
172. Soga O, Van Nostrum CF, and Hennink WE. Poly(N-(2-hydroxypropyl) methacrylamide mono/di lactate): a new class of biodegradable polymers with tuneable thermosensitivity. Biomacromolecules 5, 818-821, 2004.
173. Hennink WE, de Jong SJ, Bos GW, Veldhuis TFJ, and Van Nostrum CF. Biodegradable dextran hydrogels crosslinked by stereocomplex formation for the controlled release of pharmaceutical proteins. Int. J. Pharm. 277, 99-104, 2004.
174. Verbaan FJ, Bos GW, Oussoren C, Woodle MC, Hennink WE, and Storm G. A comparative study of different cationic transfection agents for in vivo gene delivery after intravenous administration. J. Drug Del. Sci. Tech. 14, 1-7, 2004.
175. Van Nostrum CF, Veldhuis TFJ, Bos GW, and Hennink WE. Hydrolytic degradation of oligo(lactic acid): a kinetic and mechanistic study. Polymer 45, 6779-6787, 2004.
176. Soga O, Van Nostrum CF, Ramzi A, Visser T, Soulimani F, Frederik PM, Bomans PHH, and Hennink WE. Physicochemical characterization of thermosensitive and degradable polymeric micelles. Langmuir 20, 9388-9395, 2004.
177. Stubbe BG, Hennink WE, De Smedt SC, and Demeester J. On the swelling pressure of hydrogels that degrade through different mechanisms. Macromolecules 37, 8739-8744, 2004.
178. Bos GW, Kanellos T, Crommelin DJA, Hennink WE, and Howard CR. Cationic polymers that enhance the performance of HbsAg DNA in vivo. Vaccine 23, 460-469, 2004.
179. Funhoff AM, Van Nostrum CF, Lok MC, Fretz MM, Crommelin DJA, and Hennink WE. Poly(3-guanidinopropyl methacrylate): a novel cationic polymer for gene delivery. Bioconjugate Chemistry 15, 1212-1220, 2004.
180. Verbaan FJ, Crommelin DJA, Hennink WE, and Storm G. Poly(2-dimethylamino)ethyl methacrylate)-based polymers for the delivery of genes in vitro and in vivo. In: Polymeric Gene Delivery. Ed. Malji MM. CRC Press, page 107-132, 2004.
181. Vermonde T, Van Steenbergen MJ, Besseling NAN, Marcelis ATM, Hennink WE, Sudholter EJR, and Cohen Stuart MA. Linear Rheology of water-soluble reversible

neodymium(III) coordination polymers. Journal of the American Chemical Society 126, 15802-15808, 2004.
182. Van Tomme SR, Van Steenbergen MJ, De Smedt SC, Van Nostrum CF, and Hennink WE. Self-gelling hydrogels based on oppositely charged dextran microspheres. Biomaterials 26, 2129-2135, 2005.
183. Funhoff AM, Van Nostrum CF, Lok MC, Kruijtzer JAW, Crommelin DJA, and Hennink WE. Cationic polymethacrylates with covalently linked membrane destabilizing peptides as gene delivery vectors Journal of Controlled Release 101, 233-246, 2005
184. Chung JT, Vlugt-Wensink KDF, Hennink WE, and Zhang Z. Effect of polymerization conditions on the network properties of Dex(HEMA) microspheres and macro-hydrogels. International Journal of Pharmaceutics 288, 51-61, 2005.
185. Bos GW, Hennink WE, Brouwer LA, den Otter W, Veldhuis TFJ, van Nostrum CF, and Van Luyn MJA. Biocompatibility of *in situ* formed dextran hydrogels crosslinked by stereocomplex formation after subcutaneous implantation in rats. Biomaterials 26, 3901-3909, 2005.
186. Zielhuis SW, Nijsen JFW, Figueiredo R, Feddes B, Vredenburg AM, Van het Schip AD, and Hennink WE. Surface characteristics of holmium-loaded poly(L-lactic acid) microspheres. Biomaterials 26, 925-932, 2005.

19 Fast Biodegradable Polymers

Wim E. Hennink, Jan Heln van Steenis, and Cornelus F. van Nostrum

CONTENTS

INTRODUCTION

Biodegradable polymers have found widespread application in many technological areas. They are used as biomaterials for drug delivery purposes, for example, as degradable sutures, orthopedic implants, and as scaffolds for tissue engineering applications.[1-6] These materials are not only of academic interest, but they have found commercial applications in a great number of clinical products.[8] In addition to their utility in the biomedical and pharmaceutical fields, biodegradable polymers are also used in environmentally friendly packaging materials.[10-12]

In principle polymers can be degraded via a variety of routes, including heat (pyrolysis), radiation (ultraviolet, gamma), oxidative agents (ozone, peroxides), strong acids and bases, and "simply" by water. This chapter focuses on biodegradable polymers used for biomedical and pharmaceutical applications. These polymers

0-8493-1487-9/04/$0.00+$1.50

degrade under physiological conditions almost exclusively by chem... hydrolysis of labile bonds present in the main chains, crosslinks, o... Responsive synthetic systems that react in relatively short times (sec... see other chapters in this book) to an external stimulus (temperat... well known. In contrast, degradation of polymers at 37°C triggered, ... pH, takes hours or days to months. Therefore, *rapid* biodegradability, ... chapter should be interpreted as a relative value.

The advantages of biodegradability of polymers used for the appli... tioned above are obvious. One major benefit is the fact that a biodegra... or implant does not have to be removed when it has fulfilled its intend... course, care should be taken to ensure that nontoxic degradation product... In principle, this can be guaranteed by using polymers composed of ... compounds and this explains the popularity of the poly(lactic acid) and poly... acid) families of polymers.

Biodegradability can be used to modulate the properties of systems ... control the releases of biologically active substances (drugs, pharmaceuticals, ... proteins, antigens, vaccines) from polymers. This chapter presents an over... some important classes of biodegradable polymers. Their properties, parti... their degradation characteristics, are described and strategies to modulate the ... degradability levels are discussed. Three main classes of biodegradable poly... systems are discussed, namely hydrophobic materials, insoluble but swellab... tems (hydrogels), and fully soluble polymers.

BIODEGRADABLE HYDROPHOBIC POLYMERS

Polymer Degradation: General Considerations

A great variety of biodegradable polymers (also called bioerodible polymers) ha... been developed. These polymers degrade due to hydrolytically sensitive groups b... which the monomers that form the polymer are connected with each other. During ... degradation, low molecular weight water-soluble degradation products (monomers ... and oligomers) are formed and leave the remaining solid material by diffusion. The ... degradation products are excreted via the kidneys, further degraded as oligomers, ... or metabolized as in the case of lactic acid into water and CO_2. Table 19.1 presents ... an overview of the factors that affect the degradation of polymeric materials.

Table 19.1 shows that the biodegradation rate of a material depends on several factors among which are the geometry and porosity of the degradable device.[13] The biodegradation rate also depends on typical polymer characteristics such as the type, presence of comonomers, and molecular weight.

Hydrophobic polymers with hydrolyzable bonds mainly degrade via chemical hydrolysis; enzymatic catalysis does not play a role. Since water is necessary for the degradation process, the degradation time (the time required to fully dissolve a solid material made of a biodegradable polymer) depends on the water-absorbing capacity of the material and the chemical nature of the hydrolyzable bonds. Although the materials discussed in this section are hydrophobic and therefore do not dissolve

TABLE 19.1
Factors Affecting Hydrolytic Degradation Behavior of Biodegradable Polyesters

Water permeability and solubility (hydrophilicity and hydrophobicity)
Chemical composition
Mechanism of hydrolysis (noncatalytic, autocatalytic, enzymatic)
Additives (acids, bases, monomers, solvents, drugs)
Morphology (crystalline, amorphous)
Device dimensions (size, shape, surface-to-volume ratio)
Porosity
Glass transition temperature (glassy, rubbery)
Molecular weight and molecular weight distribution
Physicochemical factors (ion exchange, ionic strength, pH)
Sterilization
Site of implantation

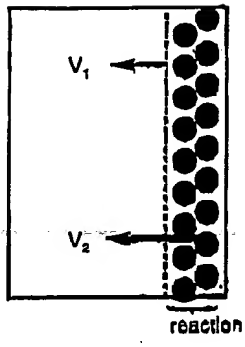
Source: Andersen, J.M., in *Biomedical Applications of Synthetic Biodegradable Polymers*, Hollinger, J.O., Ed., CRC Press, Boca Raton, FL, 1995, chap. 10.

In water, they still are able to absorb some water when placed in an aqueous environment. Generally speaking, for a certain class of related polymers (the poly(lactic acid) family) the higher the water-absorbing capacity of a material, the faster the degradation.

An important factor governing the degradation rates of polymers is the chemical nature of the bonds by which the monomers are covalently linked. As a rule, rate increases in the order of amide ← ester ← carbonate ← anhydride.[2]

Another important factor that affects biodegradation time and other characteristics of polymeric materials is the morphology of the matrix. Many degradable polymers (poly(L-lactic acid) [PLLA] and poly-ε-caprolactone) with regular structures can crystallize. In addition to a crystalline phase, these materials also have amorphous (noncrystalline) phases. The presence of a crystalline phase has large consequences for the biodegradation of materials made from these polymers. First, because crystallines are well-ordered structures with low free volumes, the water-absorbing capacity of a polymeric material decreases with increasing crystallinity. Because water is necessary for the biodegradation of polymeric materials, decreasing water absorbing capacity increases biodegradation time.

Second, the hydrolytically sensitive groups in the crystalline phase are far less susceptible to hydrolysis than the same bonds in the amorphous phase because the crystallites are essentially impermeable to water molecules. This means that in semicrystalline materials, the degradation starts in the amorphous phase. As a consequence, the crystallinity in the remaining system increases during degradation. In later stages, the crystalline regions degrade, mainly via surface erosion. The biodegradability of poly(lactic acid) and related polymers has been extensively studied especially by Vert's research group.[14–17] The lessons learned from this polymer are



V_1 = rate of water permeation
V_2 = rate of polymer hydrolysis

$V_1 > V_2$: reaction zone increases with time → bulk hyd
$V_1 < V_2$: reaction zone at surface → surface erosion

FIGURE 19.1 Bulk hydrolysis versus surface erosion. (From Anderson, J.M., in *Biom Applications of Synthetic Biodegradable Polymers*, Hollinger, J.O., Ed., CRC Press, Raton, FL, 1995, chap. 10.)

also applicable to other systems as will be discussed in more detail in the follow sections.

Degradation: Surface Erosion vs. Bulk Hydrolysis

Degradable polymers can be subdivided into different categories according to the chemical compositions. Another way to categorize them is according to their degradation behavior, namely degradation by surface erosion or bulk hydrolysis (Figure 19.1).

For the biodegradation of polymeric materials, water is necessary to hydrolyze the chemical bonds present in polymer chains. Even hydrophobic polymers absorb water when placed in an aqueous environment. Two extreme situations can be distinguished. Once the chemical hydrolysis of the labile bonds present in the degradable material is faster than the in-diffusion of water from the surrounding, surface degradation (frequently called surface erosion) occurs.[18] This degradation process is characterized by a continuous mass loss of the material over time. Moreover, the molecular weight of the polymer in the nondegraded part of the matrix does not undergo a change.

Members of the poly(anhydride) family of polymers (Figure 19.2) as developed by Langer et al.[18–21] essentially degrade via the surface erosion process. The biodegradation times of poly(anhydrides) depend on the polymer structures between the anhydride bonds. Aliphatic poly(anhydrides) degrade within days whereas aromatic systems degrade over months to years.

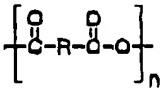


FIGURE 19.2 General structure of polyanhydride.

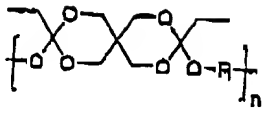


FIGURE 19.3 General structure of poly(ortho ester).

Another category of well studied materials that degrade by surface erosion are the polyorthoester systems (Figure 19.3) as designed by Heller and coworkers.[23,29] The biodegradation time of a poly(orthoester) can be controlled by polymer composition and can range from a couple days to months. The introduction of α-hydroxyacid segments is an effective method for shortening the degradation times of poly(orthoesters).[24]

Surface erodible systems are attractive for the design of controlled release systems. When a drug is homogeneously distributed over the matrix of a surface erodible system, the release of the drug essentially follows the polymer mass loss, resulting in a more or less zero order release of a drug for the duration of the degradation process.[24]

The other category of degradable materials degrades via bulk hydrolysis processes, not by surface erosion. The main group representative of such bulk degrading materials is the well-known and well-studied poly(lactic acid) family. For these materials, water penetration into the material is faster than the hydrolysis of their labile bonds (Figure 19.1). This means that when placed in an aqueous environment, these systems are saturated with water and hydrolysis consequently occurs through the homogeneous matrix. Initially, the degradation of these materials can only be determined through a decrease in molecular weight of the polymer caused by random hydrolytic cleavages of ester bonds.[25] In later stages of the degradation process, when the molecular weight has dropped to a certain level, soluble degradation products are formed which in turn are associated with mass loss.

Three points should be made regarding biomaterials that degrade via surface erosion or bulk hydrolysis. First, polymers that exclusively degrade via only one of the mechanisms do not exist in practice. In fact, polymers degrade predominantly via the surface erosion route (with some contribution of bulk degradation) and vice versa. Second, although polymeric materials initially degrade by the bulk hydrolysis mechanism, heterogeneous degradation occurs in later stages of the process, as demonstrated by Vert and coworkers for poly(lactic acid) and related polymers. The core of the material degrades faster than the shell due to accumulation of low molecular weight acidic degradation products [lactic acid (oligomers)] in the material that catalyze the hydrolysis of remaining intact ester bonds.[26] Third, PLLA is degraded by surface erosion after incubation in an aqueous solution of high pH.[27] The implication of this finding is that the degradation mechanism may change once the pH of the surroundings changes.

Degradable Aliphatic Polyesters

In principle, linear aliphatic polyesters can be obtained by polycondensation of suitable compounds having both hydroxyl groups and carboxylic acid groups or by

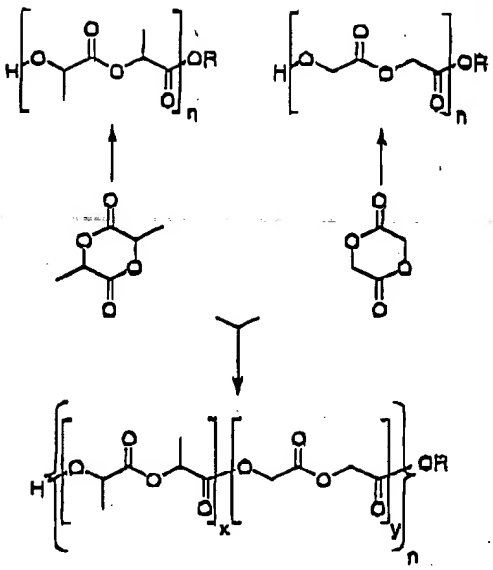


FIGURE 19.4 Synthesis of poly(lactic acid) and poly(glycolic acid) and their copolymers from cyclic precursors.

reaction of compounds having two hydroxyl groups with compounds having two carboxylic acid groups. Branched and crosslinked structures can be obtained by using trifunctional and higher functional monomers. Indeed, a great variety of linear aliphatic polyesters have been synthesized chemically.[28-30] Natural polyesters are also known. Certain microorganisms can convert glucose or other sugars into aliphatic polyesters under certain conditions, then use them as carbon and energy sources. A well-known example of these materials is poly(hydroxybutyrate, or PHB).[31,32]

An important class of hydrophobic biodegradable polyesters is the poly(lactic acid) family of polymers. Poly(lactic acid) and its copolymers with glycolic acid have very good biocompatibility and yield nontoxic endogenous compounds as degradation products.[33-35] These polymers have been studied for a variety of biomedical, biotechnological, and pharmaceutical applications.

Poly(lactic acid) can be synthesized by a polycondensation reaction of lactic acid at high temperature.[36] This route, however, yields relatively low molecular weight polymers. High molecular weight poly(lactic acid) and poly(glycolic acid) and their copolymers can be synthesized routinely by ring opening polymerization of the dilactone of lactic acid or glycolic acid using stannous 2-ethyl hexanoate or zinc powder as a catalyst (Figure 19.4).[17,37] Moreover, by synthesizing poly(α-hydroxy) acids via controlled ring opening polymerization, polymers with low polydispersities and block copolymers with well-defined structures can be produced.[38] Because poly(lactic acid) and poly(glycolic acid) are most commonly synthesized

TABLE 19.2
Biodegradation Times of Lactic Acid/Glycolic Acid Polymers

Polymer	Biodegradation Time (Months)
Poly(L-lactide)	18–24
Poly(D,L-lactide)	12–16
Poly (glycolide)	2–4
50:50 Poly(DL-lactide-co-glycolide)	2
85:15 Poly(DL-lactide-co-glycolide)	5
90:10 Poly(DL-lactide-co-caprolactone)	2

Source: From Lewis, D.H., in *Biodegradable Polymers as Drug Delivery Systems*, Chasin, M. and Langer, R., Eds., Marcel Dekker, New York, 1990. With permission.

from lactide and glycolide, respectively, their polymers are also known as polylactide and polyglycolide.

Table 19.2 summarizes the degradation times of lactic acid and glycolic acid copolymers. Since lactic acid is a chiral compound, both L and D (or S and R) forms can be distinguished. Poly(L-lactic acid) (PLLA) and poly(D-lactic acid) are rather hydrophobic semicrystalline polymers that show relatively slow biodegradation behaviors (around 18 to 24 mo).[40] The crystallinity in these polymers can be eliminated by copolymerization of L-lactide and D-lactide (or meso-lactide), yielding poly(D,L-lactic acid) — a hydrophobic amorphous material with a faster degradation time (12 to 16 mo) than PLLA. The biodegradability of poly(D,L-lactic acid) can further be modulated by copolymerization with glycolide; poly(lactic acid)-co-glycolic acid 50:50 (PL(G)A) has a degradation time of 2 mo.

This increased degradation time as compared to poly(D,L-lactic acid) arises for a number of reasons. First, the copolymer has no crystallinity, and as noted earlier, amorphous polymers degrade faster than their crystalline counterparts. Second, glycolic acid is more hydrophilic than lactic acid. This implies that the water-absorbing capacity of PL(G)A is slightly higher than that of PLLA. Higher water content results in faster hydrolytic degradation because water is the reactive species. Moreover, a higher water content leads to a higher dielectric constant in the degrading matrix, ester hydrolysis proceeds faster with increasing dielectric constant of the medium.[40] Third, the ester bond between glycolic acid and glycolic acid or lactic acid is more susceptible to degradation than an ester bond between two lactic acid molecules.[1] The rapidly degrading PL(G)A 50:50 polymer is presently under investigation for the controlled release of certain pharmaceutically active peptides and proteins.[41–43] The degradation kinetics of PL(G)A are further affected by additives such as metal salts,[44] monomers,[45] basic compounds such as corals,[46] acidic drugs,[47] superoxide ions,[48] and catalysts such as $SnOct_2$ and zinc metal.[49] To stabilize proteins in PL(G)A microspheres, additives such as $Mg(OH)_2$ have been used.[42] These additives however, may also affect the biodegradation times of PL(G)A microspheres.

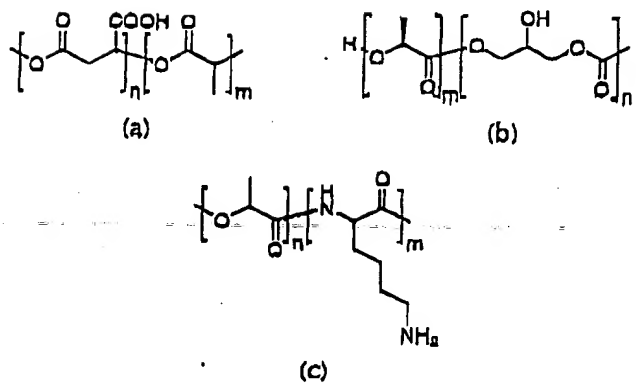


FIGURE 19.5 Biodegradable polyesters with pendant functional groups. (a) Poly(*L*-lactide-*co*-*RS*-β-malic acid). (From He, B. et al., *Polymer*, 44, 969, 2003. With permission.) (b) Poly(carbonate ester) of glycerol and lactic acid. (From Ray, W.C. and Grinstaff, M.W., *Macromolecules*, 36, 3557, 2003. With permission.) (c) Poly(lactic acid-co-lysine). (From Liu, Y. et al., *Eur. Polym. J.*, 39, 977, 2003. With permission.)

An important and obvious strategy to modulate (shorten) the biodegradation times of aliphatic polyesters is to hydrophilize these polymer systems via the introduction of functional (e.g., OH, COOH, or NH_2) side groups in the polymer chains, the introduction of hydrophilic blocks in the polymer chains, or blending with hydrophilic polymers. Functionalized polyesters have been synthesized recently[50–55] and Figure 19.5 shows some representative structures.

The properties, particularly biodegradability, of these newly synthesized polymers have not been studied in full detail yet. However, it is likely that these functionalized polyesters degrade more rapidly than their nonfunctionalized counterparts. Two reasons can be given to substantiate this hypothesis. First, functional groups increase the hydrophilicity of a polymer. Since water is necessary for hydrolytic degradation, a higher water content of polymeric material will increase degradation rate. Additionally, a higher water content in the material will result in a higher dielectric constant in the homogeneous material. In a study published recently, a higher dielectric constant of the medium caused a higher susceptibility of ester bonds for hydrolysis.[40]

Second, these hydrophilic functional groups may also act as catalysts in the degradation of ester bonds present in the polymer chains. When the content of the hydrophilic monomer exceeds a certain limit, the polymers can become fully water-soluble. These functionalized water-soluble polyesters can be used for the delivery of DNA when cationic groups are introduced or for the design of macromolecular prodrugs when carboxylic acid or hydroxyl groups present in side chains are used for the covalent attachments of drug molecules.[56]

Aliphatic polyesters can also be hydrophilized by synthesizing block or graft copolymers of PL(G)A and poly(ethylene glycol) (PEG) or dextran. In fact, the materials formed (hydrogels) have high water-absorbing capacities; their properties will be discussed in the next section.

Finally, blending is an important method of tailoring material properties to mask unfavorable characteristics and strengthen desired qualities such as improved degradability. PLLA has been blended with PEG[57] and poly(vinylpyrrolidone) (PVP).[58] PLLA is partially miscible with both PEG and PVP, resulting in increased water absorbing capacity and decreased crystallinity of the PLLA. Both factors are favorable for increasing the degradation rate of PLLA, as was observed for the PLLA and PEG blends.[57]

BIODEGRADABLE HYDROGELS

Hydrogels are polymeric networks that absorb and retain large amounts of water. In a polymeric network, the hydrophilic groups or domains present are hydrated in an aqueous environment, thereby creating a hydrogel structure. Crosslinks of a chemical or physical nature are present to prevent dissolution of the gel.[59,60] Because of their water-absorbing capacity, hydrogels are currently used for contact lenses and protein separation. To be effective in such applications, the gels must remain stable and not degrade under conditions in which they are used.

Biodegradability is advantageous for certain applications such as drug delivery.[59,60] Degradable hydrogels are often used as drug delivery materials because they are generally biocompatible and their aqueous compositions make them suitable depots for sensitive compounds such as pharmaceutically active proteins. If the size of the protein is smaller than the meshes in the hydrogel matrix, the release of the protein is governed by Fickian diffusion. In contrast, when the protein is larger than the average pores of the gel, the release is dependent on the degradation of the hydrogel.[61] The control of the degradation rate is therefore very important for adjusting the release rates of the incorporated drugs.

Long-lasting delivery systems require slow biodegradation of the hydrogel, while other applications may require a relatively fast release of drugs through rapid degradation. It is beyond the scope of this chapter to summarize the extensive literature on degradable hydrogels. We will summarize recent developments to illustrate the potentials of biodegradable hydrogels and possibilities of modulating their properties, in particular their biodegradability. The next section will cover dextran hydrogels and PEG-PL(G)A-based systems.

Hydrogel Degradation: Surface Erosion vs. Bulk Hydrolysis?

As noted earlier, the degradation of relatively hydrophobic polymers proceeds via surface erosion or bulk hydrolysis. When systems are used in their wetted forms, such as by injection or implantation of a previously hydrated gel, it is unlikely that the gels will degrade chemically via surface erosion because the network already contains a large amount of water and low molecular weight ions (e.g., hydroxyl ions) that catalyze the hydrolysis of labile bonds present in the gel can diffuse almost freely through the gel. Conversely, when a dried gel is put into an aqueous environment, in principle these materials can degrade via surface erosion. However, the dried gel will rapidly absorb water because of its hydrophilic character. Thus, in practice, hydrogels chemically degrade via a bulk hydrolysis process.

Hydrogels can also degrade by enzymatic action. Examples of such gelatins, starches, and dextran hydrogels that can be degraded by trypsin and dextranase, respectively.[59] When an enzyme is added to a proformulation surface erosion occurs. In general, the degradation time increases with concentration of enzyme and decreases with increasing crosslinking density gel, as noted in the literature concerning the degradation of crosslinked dextran by dextranase.[62]

Enzymes can also degrade hydrogels by bulk degradation. For those hydrogels matching enzyme is added to a hydrogel formulation before the crosslinking reaction is carried out. Again, the time to dissolve the gels is dependent on the amount of enzyme present in the gel and the crosslink density of the gel, as demonstrated for dextran hydrogels with entrapped dextranase.[63] When the crosslink density is too high, the enzyme is not capable of cleaving bonds in the polymer network and the gel remains stable over time.[64]

Chemically Crosslinked Dextran Hydrogels

Dextran is a naturally occurring water-soluble polysaccharide with excellent biocompatibility, used as a blood plasma expander. For that reason it is a good candidate for hydrogel preparation. One way to prepare hydrogels from water-soluble polymers is through network formation by chemical crosslinking. The method we used was aqueous free radical polymerization of dextrans modified with methacrylate groups. Dextran derivatized with methacrylate esters (dex-MA, Figure 19.6a) was obtained by reaction of the polysaccharide with glycidyl methacrylate.[65,66] In the corresponding crosslinker

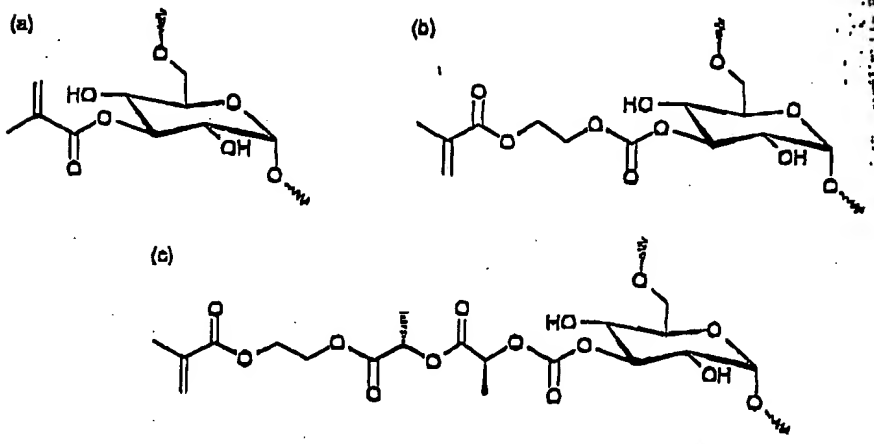


FIGURE 19.6 Polymerizable dextran derivatives used for the preparation of hydrogels. (From Van Dijk-Wolthuis, W.N.E. et al., *Macromolecules*, 30, 3411, 1997 and Van Dijk-Wolthuis, W.N.E. et al., *Macromolecules*, 30, 4639, 1997. With permission.)

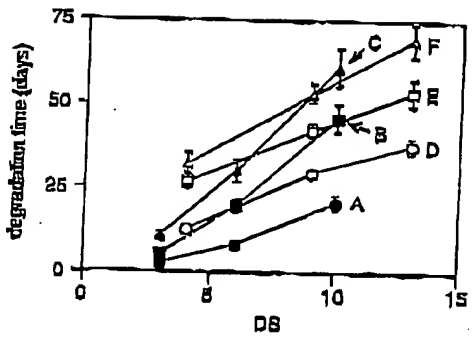


FIGURE 19.7 Degradation times of dex-HEMA (open symbols) and dex-lactate-HEMA hydrogels (closed symbols) as a function of degree of substitution (number of methacrylate groups/100 glucopyranose residues) at various initial water contents. A: dex-lactate-HEMA 90%; B: 80%; C: 70%; D: dex-HEMA 90%; E: 80%; F: 70%. (From Van Dijk-Wolthuis, W.N.E. et al., *Macromolecules*, 30, 4639, 1997. With permission.)

networks, the ester bonds appeared very stable. Under physiological conditions, dex-MA hydrogels showed few signs of degradation over a 5-month period.[67] On the other hand, when hydrolytically sensitive spacers were introduced between the methacrylate groups and the dextran backbone, degradation was significantly enhanced.

Hydrogels prepared from dextran to which 2-hydroxyethyl methacrylate units were coupled via a carbonate bond (dex-HEMA, Figure 19.6b) degraded under physiological conditions, probably due to the hydrolytical instability of the carbonate bonds. Further increase of the degradation rate was achieved by introducing additional oligolactate spacers between the HEMA units and the backbone (dex-lactate-HEMA, Figure 19.6c). As shown in Figure 19.7, the degradation time of dex-lactate-HEMA can be fine-tuned by the degree of substitution of dextran and the initial water content of the hydrogels from 2 days to 2 months.[67] As a consequence, the release rates of encapsulated proteins can be tailored to a similar extent.

Physically Crosslinked Dextran Hydrogels

Crosslinking of polymers to form hydrogels by chemical means has the disadvantage that the required conditions may affect the encapsulated drugs. This is especially the case when highly sensitive biomolecules such as proteins and genes are the therapeutic compounds. Network formation by physical interactions between groups or segments of polymers is favored.[40] We applied the concept of stereocomplex formation to create physical crosslinks in biodegradable dextran hydrogels. Dextran was substituted with oligolactic acid side chains containing L-lactic acid (dex-L-OLA) or D-lactic acid enantiomers (dex-D-OLA; Figure 19.8).

These graft copolymers are soluble in water when short OLA grafts are attached to the dextran backbone. Interestingly, when aqueous solutions of dex-L-OLA and dex-D-OLA with minimum chain lengths of 11 lactic acid units are mixed, the L-OLA and D-OLA grafts associate to form so-called stereocomplex crystallites

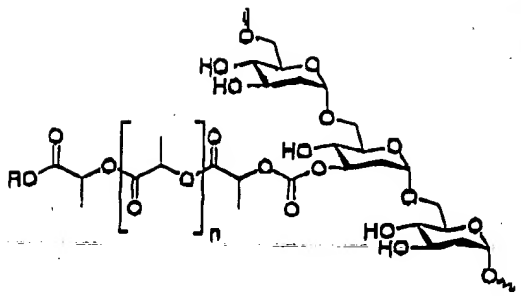


FIGURE 19.8 Dextran grafted with oligolactate chains. (From De Jong, S.J. et al., *Macromolecules*, 33, 3680, 2000. With permission.)

and viscoelastic gels. These hydrogels are thermoreversible; they can be dissolved and reconstructed upon heating and cooling, respectively.[68] Degradation of the dex-OLA gels occurs through hydrolysis of the OLA grafts. After an initial swelling phase, the gels fully degraded in 1 to 7 d, depending on the degree of substitution of dextran and the initial water contents of the gels.[69]

A more detailed investigation of the degradation of one of the single enantiomers (dex-L-OLA) revealed that the amount of lactic acid still grafted to the backbone decreased with a half-life of about 2 days (physiological conditions). Mass spectrometry analysis of the degradation products suggested that the carbonate bond connecting the dextran with the lactic acid oligomer was hydrolyzed first. This may be attributed to the neighboring dextran hydroxy groups that stabilize the transition state during hydrolysis. This phenomenon results in the removal of a complete side chain that then further degrades to smaller oligomers.[69] HPLC analysis showed that the degradation of the latter oligomers proceeded stepwise from their hydroxy termini with half-lives of a couple hours at pH 7. One lactic acid dimer was split off in each step by a process called backbiting.[40]

Degradable Hydrogels Based on Amphiphilic Copolymers of PL(G)A and PEG

Blockcopolymers of PEG and PL(G)LA (Figure 19.9 shows a representative structure) were synthesized with the aim of using the polymers for the delivery of certain pharmaceutically active proteins.[70–72]

PEG was introduced to increase the water-absorbing capacity of PL(G)A systems. By controlling the water content, the intention was to simultaneously control the compatibility with entrapped proteins, their release rates, and the degradation

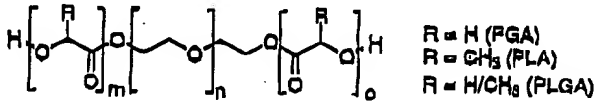


FIGURE 19.9 A-B-A triblock copolymer of PEG and poly(α-hydroxy acids).

behavior of the PEG-PL(G)A hydrogels. Other hydrophilic polymers such as poly(vinyl alcohol)[73] were combined with PL(G)A; PEG was combined with other aliphatic polyesters such as poly(ε-caprolactone)[74] and poly(R-3-hydroxy butyrate)[75] with the same goal in mind. ABA block copolymers (Figure 19.9) of PLA (A block) and PEG (B block) were soluble in water when the degree of polymerization (DP) $DP_{PEG}/DP_{PLA} > 4$.[76] When this ratio was <4, turbid aqueous solutions were obtained, likely due to the formation of micelles.[76]

ABA block copolymers of PL(G)A (A block) and PEG (B block) with long PL(G)A blocks were soluble in typical solvents for PL(G)A-like dichloromethane and chloroform. Drug-loaded systems based on these triblock copolymers were prepared essentially using the same methods (e.g., solvent evaporation) developed for the preparation of PL(G)A microspheres.[71,77–78] The research on the degradation behavior of hydrogels based on PL(G)A-PEG-PL(G)A blockcopolymers has been summarized by Kissel et al.[72] The degradation of these polymers was unpredictable because the degradation behavior of PL(G)A is complex and because of the complicated phase behavior of these systems. No general rules on the degradation of PL(G)A-PEG-PL(G)A have been devised yet.

PLA-PEG-PLA and PGA-PEG-PGA are soluble in water and consequently do not form hydrogels when the degree of polymerization of the polyester chains is <5 and the molecular weight of the PEG block is 1 to 20 kDa.[76] Hydrogels based on these blockcopolymers were obtained by the reaction of the hydroxyl termini with acryloyl chloride followed by a radical polymerization of the acrylated PLLA-PEG-PLLA polymers.[80] The degradation times of the gels obtained under physiological conditions were dependent on composition and varied from 1 to 120 d. Generally speaking, the longer the PEG chains, the less dense the network and the shorter the degradation time. Further, systems with PGA segments degraded faster than comparable systems with PLA. The degradation behaviors of these gels were modeled by Martens and Metters et al. and the developed models clearly describe the observed degradation and mass loss profiles.[81,82]

A major breakthrough was realized after it was found that PEG-PL(G)A-PEG block copolymers were soluble in water at room temperature and solidified when brought to 37°C.[83,84] This sol–gel behavior depended on the molecular weights of the PEG and PL(G)A blocks and the polymer concentration in water.[84,85] This behavior makes these systems very attractive for biodegradable injectable *in situ* drug delivery systems.[84,86] No detailed degradation studies have been reported yet. The PEG-PL(G)A-PEG copolymers are, as expected, degradable and have half-lives around 30 days *in vivo*.[86] No structure–biodegradability relationships have been reported.

BIODEGRADABLE SOLUBLE POLYMERS

Water Soluble Polymers for Tumor Targeting

Water-soluble polymers can be degraded enzymatically [polymers based on natural poly(α-amino acids)][87,88] or chemically. Water-soluble, nondegradable polymers can be removed from the circulation by excretion via the kidneys if the molecular weight

is not too high (e.g., <40 kDa for dextran).[89] Soluble polymers are pres... investigation as targeted delivery systems, for example, for cytostatic ... drugs are covalently linked to polymers via degradable linkers.

Worth mentioning is the important work done by Kopecek, Ulbrich, and ... on poly(N-(2-hydroxypropyl)methacrylamide)–doxorubicin conjugates.[90,91] ... polymers are designed in such a way that they are stable in the general ci... and degraded in the endosomal and lysosomal compartments of tumor cel... process liberates (releases) the polymer-bound drug by interpolating a peptid... between the polymer backbone and doxorubicin that is specifically cleav... proteases present in the cell organelles. As a result, a high concentration ... cytostatic drug is delivered intracellularly, but not outside the cells. Thus, th... effects of the toxic drug are minimized. Tumor cell recognition and uptake ... polymeric prodrug are promoted by the coupling of a targeting ligand [monoclo... antibodies (fragments), galactose] to the polymer–drug conjugate.[36]

In a more recent approach, doxorubicin was coupled to the polymer back... via a hydrazone bond that was stable under physiological conditions (pH 7.4) ... hydrolytically degraded in a mild acidic environment.[92] This means that the dr... was released once the polymer–drug conjugate entered the endosomal and lysosomal compartments of cells. The advantage of this chemically degrading system over ... enzymatically degrading polymeric prodrug is that release of the drug is independe... of proteolytic activity in the endosomes.[89]

Degradable Polymers for Gene Delivery

Gene therapy has been proposed to treat diseases such as cystic fibrosis that originate from inherited genetic deficiencies and also to treat acquired genetic disorders such as cancer, cardiovascular disease, and rheumatoid arthritis.[93,94] In order to express the exogenous gene, the DNA must be delivered into the nucleus of the target cell. Since DNA is a large hydrophilic molecule with an overall negative charge, it does not easily pass through cellular membranes. Furthermore, DNA must be protected from degradation by deoxyribonucleases (DNases).

To obtain acceptable gene expression levels, the use of a carrier, e.g., a cationic polymer, is required to bring the plasmid into the target cell.[95] Known polymeric carriers such as polyethylenimine (pEI), poly(2-dimethylaminoethyl methacrylate) (pDMAEMA), and poly-L-lysine (pLL), are nonbiodegradable (pEI and pDMAEMA) or show low transfection activity (pLL).[96,97]

Considerable efforts have been made in recent years to design biodegradable polycations that can be used as synthetic DNA carriers. The use of degradable carriers would allow controlled release of encapsulated DNA after it is taken up by target tissues or cells, followed by subsequent metabolism and excretion of the carrier. A sufficiently long lifetime of the carrier, i.e., a few hours, is required in order to allow the polymer–DNA complexes to reach the target sites unaffected after intravenous or other administration. However, to deliver and release DNA efficiently into cells, a carrier should preferably be degraded within a few days.

The use of degradable polyesters as gene delivery systems has some advantages, since they form nontoxic degradation products. For example, PL(G)A microspheres

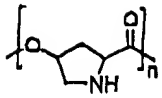


FIGURE 19.10 Poly(4-hydroxy-L-proline ester).

have been used as gene carriers, but their slow degradation rates may limit their applicability in gene therapy.[98] Increasing the hydrophilicity of polyesters by using hydrophilic comonomers is a frequently exploited strategy to accelerate hydrolytic degradation (see the section discussing biodegradable aliphatic polyesters).

Among the most rapidly degrading polyesters are those substituted with carboxylic acid or amine groups. For example, a 90:10 copolymer of lactic acid and α-malic acid degraded within 1 wk at pH 7.2 and 37°C.[99] The influence of amine substitution was shown by Langer et al., who compared the degradation of poly(lactic acid) and poly(lactic acid-co-lysine). The latter polyester appeared to degrade to half its molecular weight after 5 wk (pH 7.1 and 37°C), whereas poly(lactic acid) took 15 weeks under the same conditions to degrade to the same extent.[100] Thus, water-soluble polyamine polyesters may be potentially useful for the delivery of genes because they can form polyion complexes with DNA and may degrade rapidly.

Poly(4-hydroxy-L-proline ester) (PHP; Figure 19.10) was the first water-soluble polycationic biodegradable polymer to be used as a gene carrier, as reported independently by Park et al.[101] and Langer et al.[102]

The degradation of the polymer in aqueous solution was monitored at pH 7 and 37°C using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. The polymer showed rapid initial degradation to less than half the molecular weight of intact polymer in less than 2 h. However, due to the formation of acid degradation products, hydrolysis slowed down after the initial degradation phase by decreasing pH of the solution; complete degradation to monomeric units took about 3 mo. PHP was able to bind DNA and protect it against degradation by nucleases for at least 4 h.[101] This indicates that PHP in the complex is not degraded as quickly as when the polymer is not bound to DNA. Poly(α-(4-aminobutyl)-L-glycolic acid) (PAGA; Figure 19.11) is an amine-substituted polyester synthesized by Kim et al.[103,104]

PAGA showed similar degradation behavior as compared to PHP, displaying a rapid initial degradation within 100 min to one third of its starting molecular weight, followed by gradual hydrolysis to the monomers in 6 mo at 37°C.[104] The rapid

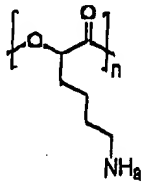


FIGURE 19.11 Poly(α-(4-aminobutyl)-L-glycolic acid) (PAGA).

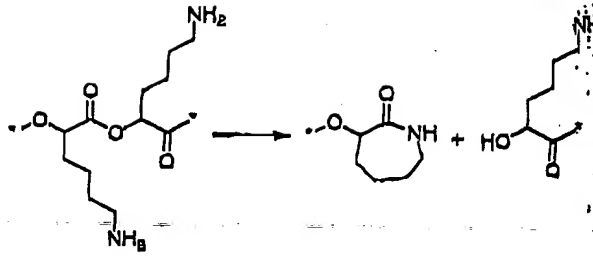


FIGURE 19.12 Intramolecular degradation of PAGA.

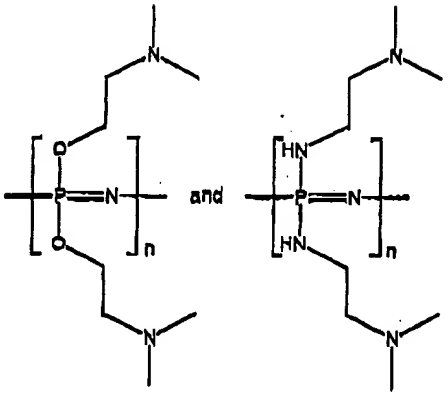


FIGURE 19.13 Cationic water-soluble polyphosphazenes. (From Luten, J. et al., *J. Control. Rel.*, 89, 483, 2003. With permission.)

hydrolysis of PHP and PAGA is attributed to the secondary amine groups in each monomeric unit that act as nucleophiles, probably by the mechanism as shown in Figure 19.12.

In line with the results from PHP, Kim et al. also observed slower degradation of the PAGA:DNA complex than the polymer alone; the complexes were stable for 8 h and dissociated completely in 1 day — a favorable timeframe for *in vivo* gene delivery. As compared with the polyamide analogue of PAGA (polylysine), the polyester was shown to be a more efficient gene delivery vector by *in vitro* tests and displayed less cytotoxicity.[104] This result illustrates the favorable effect of increased biodegradability of a carrier. Successful *in vivo* animal studies have been carried out recently.[105] In our laboratory, cationic polyphosphazenes (Figure 19.13) were synthesized and evaluated as gene delivery systems.[106]

In vitro studies showed that complexes of these polymers with plasmid DNA were able to transfect cells. The cytotoxicities of these polymers were less than those of other polymeric transfectants. The polymers were degradable at physiological conditions (half-life of 5 to 8 days at pH 7.2 and 37°C). However, to use polymer

degradation as a tool to release DNA intracellularly, more rapidly degrading polymers are required. Current investigations are aimed at improving the degradation rates of the polymers and involve the synthesis of polyphosphazenes with different amine-bearing side groups and hydrolysis-sensitive cosubstituents.

CONCLUDING REMARKS

When designing fast degrading polymers and hydrogels, one must consider several parameters including the intrinsic hydrolytic sensitivity of the bonds, the extent of hydration of the polymers, and the presence of functional groups in the neighborhood of the labile bonds that may accelerate their degradation. Several examples of the latter phenomenon have been discussed in this chapter, including polyesters containing carboxylic and amine side groups, dextran hydrogels, and lactic acid oligomers whose degradation is influenced by neighboring hydroxyl groups. Suggested mechanisms of degradation enhancement by these functional groups can range from nucleophilic attack by amine groups to stabilization of the transition state by hydroxyl groups. These insights should, in principle, make it possible to modulate and tailor the biodegradation behaviors of polymeric materials.

REFERENCES

1. Uhrich, K.E., Cannizzaro, S.M., Langer, R.S., and Shakesheff, K.M., Polymeric systems for controlled drug release, *Chem. Rev.*, 99, 3181–3198, 1999.
2. Gombotz, W.R. and Pettit, D.K., Biodegradable polymers for protein and peptide drug delivery, *Bioconjugate Chem.*, 6, 332–351, 1995.
3. Jagur-Grodzinski, J., Biomedical applications of polymers, *e-Polymers*, 12, 1–38, 2003.
4. Gupta, P., Vermani, K., and Garg, S., Hydrogels: from controlled release to pH-responsive drug delivery, *Drug Discovery Today*, 7, 569–579, 2002.
5. Weller, A., Hoffmann, R.F.G, Stahelin, A.C., Helling, H.J., and Sudkamp, N.P., Biodegradable implants in sports medicine: the biological base, *Arthroscopy*, 16, 305–321, 2000.
6. Bostman, O. and Pihlajamaki H., Clinical biocompatibility of biodegradable orthopaedic implants for internal fixation: a review, *Biomaterials*, 21, 2615–2621, 2000.
7. Agrawal, C.M. and Ray, R.B., Biodegradable polymeric scaffolds for musculoskeletal tissue engineering, *J. Biomed. Mater. Res.*, 55, 141–150, 2001.
8. Woodfield, T.B.F., Bezemer, J.M., Pieper, J.S., Van Blitterswijk, C.A., and Riesle, J., Scaffolds for tissue engineering of cartilage, *Crit. Rev. Eukaryot. Gene Expr.*, 12, 209–236, 2002.
9. Ueda, H. and Tabata, Y., Polyhydroxyalkonate derivatives in current clinical applications and trials, *Adv. Drug Del. Rev.*, 55, 501–518, 2003.
10. Van der Walle, G.A., De Koning, G.J., Weusthuis, R.A., and Eggink, G., Properties, modifications and applications of biopolyesters, *Adv. Biochem. Eng. Biotechnol.*, 71, 263–291, 2001.
11. Weber, C.J., Haugaard, V., Festersen, R. and Bertelsen, G., Production and applications of biobased packaging materials for the food industry, *Food Addit. Contam.*, 19, 172–177, 2002.

12. Pagga, U., Compostable packaging materials: test methods and l... biodegradation, *Appl. Microbiol. Biotechnol.*, 51, 125–133, 1999.
13. Anderson, J.M., Perspectives on the *in vivo* responses of biodegradable ... *Biomedical Applications of Synthetic Biodegradable Polymers*, Hollinger, ... CRC Press, Boca Raton, FL, 1995, chap. 10.
14. Li, S.M., Garreau, H., and Vert, M., Structure–property relationships in ... the degradation of massive poly(alpha-hydroxacids) in aqueous media. 3. ... of the morphology of poly(L-lactic acid), *J. Mater. Sci. Mater. Med.*, 1, ... 1990.
15. Vert, M., Li, S., and Garreau, H., More about the degradation of La/G... matrices in aqueous media, *J. Control. Rel.*, 16, 15–26, 1991.
16. Vert, M., Mauduit, J., and Li. S., Biodegradation of PLA/GA polymers: ... complexity, *Biomaterials*, 15, 1209–1213, 1994.
17. Vert. M., Schwach, G., Engel, R., and Coudane, J., Something new in the ... PLA/GA bioresorbable polymers? *J. Control. Rel.*, 53, 85–92, 1998.
18. Siepmann, J. and Gopferich, A., Mathematical modeling of bioerodible, poly... drug delivery systems, *Adv. Drug Del. Rev.*, 48, 229–247, 2001.
19. Gopferich, A. and Tessmar, J., Polyanhydride degradation and erosion, *Adv. ... Del. Rev.*, 54, 911–931, 2002.
20. Wang, P.P., Frazier, J., and Brem, H., Local drug delivery to the brain, *Adv. D... Del. Rev.*, 54, 987–1013, 2002.
21. Leong, K.W., Brott, B.C., and Langer, R., Bioerodible polyanhydrides as drug-car... matrices. I. Characterization, degradation, and release characteristics, *J. Biom... Mater. Res.*, 19, 941–955, 1985.
22. Heller, J., Barr, J., Ng, S.Y., Shen, H.R., Schwach-Abdelaoui, K.S., Rothen-Weinhold, A., and Gurny, R., Poly(orthoesters): their development and some recent applications, *Eur. J. Pharm. Biopharm.*, 50, 121–128, 2000.
23. Heller, J., Barr, J., Ng, S.Y., Abdelaoui, K.S., and Gurny, R., Poly(orthoesters): synthesis, characterization, properties and uses, *Adv. Drug Del. Rev.*, 54, 1015–1039, 2002.
24. Ng, S.Y., Shen, H.R., Lopez, E., Zhershin, Y., Barr, J., Schacht, E., and Heller, J., Development of a poly(orthoester) prototype with a latent acid in the polymer backbone for 5-fluorouracil delivery, *J. Control. Rel.*, 65, 367–374, 2000.
25. Pitt, C.G., Gratzl, M.M., Kimmel, G.L., Surles, J., and Schindler, A., Aliphatic polyesters. II. The degradation of poly(D,L)-lactide), poly(epsilon-caprolactone), and their copolymers *in vivo*, *Biomaterials*, 2, 215–220, 1981.
26. Therin, M., Christel, P., Li, S., Garreau, H., and Vert, M., *In vivo* degradation of massive poly(α-hydroxy acids): validation of *in vitro* findings, *Biomaterials*, 13, 594–600, 1992.
27. Von Burkersroda, F., Schedl, L., and Gopferich, A., Why degradable polymers undergo surface erosion or bulk erosion, *Biomaterials*, 23, 4221–4231, 2002.
28. Okada, M., Chemical syntheses of biodegradable polymers, *Prog. Polym. Sci.*, 27, 87–133, 2002.
29. Sodergard, A. and Stolt, M., Properties of lactic acid based polymers and their correlation with composition, *Progr. Polym. Sci.*, 27, 1123–1163, 2002.
30. Edlund, U. and Albertsson, A.C., Polyesters based on diacid monomers, *Adv. Drug Del. Rev.*, 55, 585–609, 2003.
31. Kim, Y.B. and Lenz, R.W., Polyesters from microorganisms, *Adv. Biochem. Eng. Biotechnol.*, 71, 51–79, 2001.

32. Lee, S.Y. and Choi, J.I., Production of microbial polyester by fermentation of recombinant microorganisms, *Adv. Biochem. Eng. Biotechnol.*, 71, 183–207, 2001.
33. Shive, M.S. and Anderson, J.M., Biodegradation and biocompatibility of PLA and PL(G)A microspheres, *Adv. Drug Del. Rev.*, 28, 5–24, 1997.
34. Athanasiou, K.A., Niederauer, G.G., and Agrawal, C.M., Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers, *Biomaterials*, 17, 93–102, 1996.
35. Cadée, J.A., Brouwer, L.A., Den Otter, W., Hennink, W.E., and Van Luyn, M.J.A., A comparative biocompatibility study of microspheres based on crosslinked dextran or poly(lactic-co-glycolic)acid after subcutaneous injection in rats, *J. Biomed. Mater. Res.*, 56, 600–609, 2001.
36. Hyon, S.H., Jamshidi, K., and Ikada, Y., Synthesis of polylactides with different molecular weights, *Biomaterials*, 18, 1503–1508, 1997.
37. Loenslag, J.W. and Pennings, A.J., Synthesis of high-molecular-weight poly(L-lactide) initiated with tin 2-ethylhexanoate, *Makromol. Chem.*, 188, 1809–1814, 1987.
38. Zhong, Z., Dijkstra, P.J., Brig, C., Westerhausen, M., and Feijen, J., A novel and versatile calcium-based initiator system for the ring-opening polymerization of cyclic esters, *Macromolecules*, 34, 3863–3868, 2001.
39. Lewis, D.H., Controlled release of bioactive agents from lactide/glycolide polymers, in *Biodegradable Polymers as Drug Delivery Systems*, Chasin, M. and Langer, R., Eds., Marcel Dekker, New York, 1990.
40. De Jong, S.J., Arias, E.R., Rijkers, D.T.S., Van Nostrum, C.F., Kettenes-van den Bosch, J.J., and Hennink, W.E., New insights into the hydrolytic degradation of (poly(lactic acid): participation of the alcohol terminus, *Polymer*, 42, 2795–2802, 2001.
41. Van de Weert, M., Hennink, W.E., and Jiskoot, W., Protein instability in poly(lactic-co-glycolic acid) microparticles, *Pharm. Res.*, 17, 1159–1167, 2000.
42. Schwendeman, S.P., Recent advances in the stabilization of proteins encapsulated in injectable PL(G)A delivery systems, *Crit. Rev. Ther. Drug Carr. Syst.*, 19, 73–98, 2002.
43. Johansen, P., Men, Y., Merkle, H.P., and Gander, B., Revisiting PLA/PL(G)A microspheres: an analysis of their potential in parenteral vaccination, *Eur. J. Pharm. Biopharm.*, 50, 129–146, 2000.
44. Zhang, Y., Zale, S., Sawyer, L., and Bornstein, H.J., Effects of metal salts on poly(DL-lactide-co-glycolide) polymer hydrolysis, *J. Biomed. Mater. Res.*, 34, 531–538, 1997.
45. Nakamura, T., Hitomi, S., Watanabe, S., Shimizu, Y., Jamshidi, K., Hyon, S.-H., and Ikada, Y., Bioabsorption of polylactides with different molecular properties, *J. Biomed. Mater. Res.* 23, 1115–1130, 1989.
46. Li, S., Girod-Holland, S., and Vert, M., Hydrolytic degradation of poly(DL-lactic acid) in the presence of caffeine base, *J. Control. Rel.*, 40, 40–53, 1996.
47. Juni, K., Ogata, J., Matsui, N., Kubota, M., and Nakano, M., Modification of the release rate of aclarubicin from poly(lactic acid) microspheres by using additives, *Chem. Pharm. Bull.*, 33, 1734–1738, 1985.
48. Lee, K.H., Won, C.Y., Chu, C.C., and Gitsov, I., Hydrolysis of biodegradable polymers by superoxide ions, *J. Polym. Sci. A Polym. Chem.*, 37, 3558–3567, 1999.
49. Swach, G., Coudance, J., Engel, R., and Vert, M., More about the polymerisation of lactides in the presence of stannous octoate, *J. Polym. Sci. A Polym. Chem.*, 35, 3431–3440, 1997.
50. Lou, X.D., Detrembleur, C., and Jerome, R., Novel aliphatic polyesters based on functional cyclic (di)ester, *Macromol. Rapid Commun.*, 24, 161–172, 2003.

51. He, B., Bei, J.Z., and Wang, S.G., Synthesis and characterization of a biodegradable copolymer: poly(L-lactide-co-RS-β-malic acid), *Polymer*, 2003.
52. Ray, W.C. and Grinstaff, M.W., Polycarbonate and poly(carbonate-ester) from biocompatible building blocks of glycerol and lactic acid, *Macromol.* 3557–3562, 2003.
53. Liu, Y., Yuan, M.L., and Deng, X.M., Study on biodegradable polymeric and characterization of poly(DL-lactic acid-co-L-lysine) random copoly *Polym. J.*, 39, 977–983, 2003.
54. Zhang, S.P., Yang, J., Liu, X.Y., Chang, J.H., and Cao, A.M., Synthesis and terization of poly(butylene succinate-co-butylene malate): a new biode copolyester bearing hydroxyl pendant groups, *Biomacromolecules*, 4, 437–44
55. Stigers, D.J. and Tew, G.N., Poly(3-hydroxyalkanoates)s functionalized with ylic acid groups in the side chain, *Biomacromolecules*, 4, 193–195, 2003.
56. Kopecek, J., Kopeckova, P., Minko, T., Lu, Z.R., and Peterson, C.M., Water polymers in tumor targeted delivery, *J. Control. Rel.*, 74, 147–158, 2001.
57. Nijenhuis, A.J., Colstee, E., Grijpma, D.W., and Pennings, A.J., High mol weight poly(L-lactide) and poly(ethylene oxide) blends: thermal characterizati physical properties, *Polymer*, 37, 5849–5857, 1996.
58. Zhang, G.B., Zhang, J.M., Zhou, X.S., and Shen, D.Y., Miscibility and phase str of binary blends of polylactide and poly(vinylpyrrolidone), *J. Appl. Polym. Sci.* 973–979, 2003.
59. Park, K., Shalaby, W.S.W., and Park, H., *Biodegradable Hydrogels for Drug Deli* Technomic Publishing, Basel, 1993.
60. Hennink, W.E. and Van Nostrum, C.F., Novel crosslinking methods to design hyd gels, *Adv. Drug Del. Rev.*, 54, 13–36, 2002.
61. Hennink, W.E., Franssen, O., Van Dijk-Wolthuis, W.N.E., and Talsma, H., Dextran hydrogels for the controlled release of proteins, *J. Control. Rel.*, 48, 107–114, 1997.
62. Simonsen, L., Hovgaard, L., Mortensen, P.B., and Bronsted, H., Dextran hydrogels for colon-specific drug delivery. V. Degradation in human intestinal incubation models, *Eur. J. Pharm. Sci.*, 3, 329–337, 1995.
63. Franssen, O., Vos, O.P., and Hennink, W.E., Delayed release of a model protein from enzymatically degrading dextran hydrogels, *J. Contr. Rel.*, 44, 237–245, 1997.
64. Franssen, O., Van Rooijen, R.D., De Boer, D., Maes, R.A.A., and Hennink, W.E., Enzymatic degradation of crosslinked dextrans, *Macromolecules*, 32, 2896–2902, 1999.
65. Van Dijk-Wolthuis, W.N.E., Franssen, O., Talsma, H., Van Steenbergen, M.J., Kettenes-van den Bosch, J.J., and Hennink, W.E., Synthesis, characterization and polymerization of glycidyl methacrylate derivatized dextran, *Macromolecules*, 28, 6317–6322, 1995.
66. Van Dijk-Wolthuis, W.N.E., Kettenes-van den Bosch, J.J., Van der Kerk-van Hoof, A., and Hennink, W.E., Reaction of dextran with glycidyl methacrylate: an unexpected transesterification, *Macromolecules*, 30, 3411–3413, 1997.
67. Van Dijk-Wolthuis, W.N.E., Hoogeboom, J.A.M., Van Steenbergen, M.J., Tsang, S.K.Y., and Hennink, W.E., Degradation and release behaviour of dextran-based hydrogels, *Macromolecules*, 30, 4639–4645, 1997.
68. De Jong, S.J., De Smedt, S.C., Wahls, M.W.C., Demeester, J., Kettenes-van den Bosch, J.J., and Hennink, W.E., Novel self-assembled hydrogels by stereocomplex formation in aqueous solution of enantiomeric lactic acid oligomers grafted to dextran, *Macromolecules*, 33, 3680–3686, 2000.

69. De Jong, S.J., Van Eerdenbrugh, B., Van Nostrum, C.R, Kettenes-van den Bosch, J.J., and Hennink, W.E., Physically crosslinked dextran hydrogels by stereocomplex formation of lactic acid oligomers: degradation and protein release behavior, *J. Control. Rel.*, 71, 261–275, 2001.
70. Li, Y.X. and Kissel, T., Synthesis and properties of biodegradable ABA triblock copolymers consisting of poly(L-lactic acid) or poly(L-lactic-co-glycolic acid) A-blocks attached to central poly(oxyethylene) B-blocks, *J. Control. Rel.*, 27, 247–257, 1993.
71. Li, Y.X., Volland, C., and Kissel, T., *In vitro* degradation and bovine serum albumin release of the ABA triblock copolymers consisting of poly(L(+) lactic acid) or poly(L(+) lactic acid-co-glycolic acid) A-blocks attached to central polyoxyethylene B-blocks, *J. Control. Rel.*, 32, 121–128, 1994.
72. Kissel, T., Li, Y.X., and Unger, F., ABA-triblock copolymers from biodegradable polyester A-blocks and hydrophilic poly(ethylene oxide) B-blocks as a candidate for *in situ* forming hydrogel delivery systems for proteins, *Adv. Drug Del. Rev.*, 54, 99–134, 2002.
73. Pistel, K.F., Breitenbach, A., Zhange-Volland, R., and Kissel, T., Brush-like branched biodegradable polyesters. III. Protein release from microspheres of poly(vinyl alcohol)-graft-poly(D,L-lactic-co-glycolic acid), *J. Control. Rel.*, 73, 7–20, 2001.
74. Martini, L., Attwood, D., Collett, J.H., Nicholas, C.V., Tanodekaew, S., Deng, N.J., Hearly, F., and Booth, C., Micellization and gelation of triblock copolymer of ethylene oxide and epsilon-caprolactone, $Cl_nE_mCl_p$, in aqueous solution, *J. Chem. Soc. Faraday Trans.*, 90, 1961–1966, 1994.
75. Li, X., Li, J., and Leong, L.W., Preparation and characterization of inclusion complexes of biodegradable amphiphilic poly(ethylene oxide) triblock copolymers with cyclodextrins, *Macromolecules*, 36, 1209–1214, 2003.
76. Rashkov, I., Manolova, N., Li, S.M., Espartero, J.L., and Vert, M., Synthesis, characterization and hydrolytic degradation of PLA/PEO/PLA triblock copolymers with short poly(L-lactic acid) chains, *Macromolecules*, 29, 50–56, 1996.
77. Kissel, T., Li, Y.X., Volland, C., Gorich, S., and Koneberg, R., Parenteral protein delivery systems using biodegradable polyesters of ABA block structure, containing hydrophobic poly(lactic-co-glycolic) A blocks and hydrophilic poly(ethylene oxide) B blocks, *J. Control. Rel.*, 39, 315–326, 1996.
78. Jain, R.A., The manufacturing techniques of various drug-loaded biodegradable poly(lactide-co-glycolide) (PL(G)A) devices, *Biomaterials*, 21, 2475–2490, 2000.
79. Zhou, S., Liao, X., Li, X., Deng, X., and Li, H., Poly-D,L-lactide-co-poly(ethylene glycol) microspheres as potential vaccine delivery systems, *J. Control. Rel.*, 86, 195–205, 2003.
80. Sawhney, A.S., Pathak, C.P., and Hubbell, J.A., Bioerodible hydrogels based on photopolymerized poly(ethyleneglycol)-co-poly(alpha-hydroxy acid) diacrylate macromers, *Macromolecules*, 26, 581–587, 1993.
81. Martens, P., Metters, A.T., Anseth, K.S., and Bowman, C.N., A generalized bulk-degradation model for hydrogel networks formed from multivinyl cross-linking molecules, *J. Phys. Chem. B*, 105, 5131–5138, 2001.
82. Metters, A.T., Anseth, K.S., and Bowman, C.N., A statistical kinetic model for the bulk degradation of PLA-b-PEG-b-PLA hydrogel networks: Incorporating network non-idealities, *J. Phys. Chem. B*, 105, 8069–8076, 2001.
83. Jeong, B., Bae, Y.H., Lee, D.S., and Kim, S.W., Biodegradable block copolymers as injectable drug-delivery systems, *Nature*, 388, 860–862, 1997.

84. Jeong, B., Bae, Y.H., and Kim, S.W., Drug release from biodegradable thermosensitive hydrogel of PEG-PL(G)A-PEG triblock copolymers, *J. Co* 63, 155–163, 2000.
85. Kwon, K.W., Park, M.J., Bae, Y.H., Kim, H.D., and Char, K., Gelation b PEG-PL(G)A-PEG triblock copolymers in water, *Polymer*, 43, 3353–3358
86. Jeong, B., Bae, Y.H., and Kim, S.W., *In situ* gelation of PEG-PL(G)A-PEG copolymer aqueous solutions and degradation thereof, *J. Biomed. Mater.* 171–177, 2000.
87. Rypacek, F., Pytela, J., Kotva, R., Skarda, V., and Cifkova, I., Biodegrada poly(amino acid)s: evaluation methods and structure–function-relationships, *mol. Symp.*, 123, 9–24, 1997.
88. Chiu, H.C., Kopeckova, P., Deshmane, S.S., and Kopecek, J., Lysosomal degrad of poly(α-amino acids), *J. Biomed. Mater. Res.*, 34, 381–392, 1997.
89. Mehvar, R. and Shepard, T., Molecular weight-dependent pharmacokinetics of rescein-labeled dextrans in rats, *J. Pharm. Sci.*, 81, 908–912, 1992.
90. Duncan, R., Polymer conjugates for tumour targeting and intracytoplasmatic deli the EPR effect as a common gateway? *Pharm. Sci. Technol. Today*, 2, 441–449, 1
91. Kopecek, J., Kopeckova, P., Minko, T., and Lu, Z.R., HPMA copolymer–antican drug conjugates: design, activity and mechanism of action, *Eur. J. Pharm. Biopharm.* 50, 61–81, 2000.
92. Rihova, B., Etrych, T., Pechar, M., Jelinkova, M., Strasmy, M., Hovorka, O., Kov M., and Ulbrich, K., Doxorubicin bound to HPMA copolymer carrier through hydra zone bond is effective also in a cancer cell line with a limited content of lysosomes, *J. Control. Rel.*, 74, 225–232, 2001.
93. Blaese, R.M., Gene therapy for cancer, *Sci. Am.*, 6, 91–95, 1997.
94. Prud'homme, G.J., Gene therapy of autoimmune diseases with vectors encoding regulatory cytokines or inflammatory cytokine inhibitors, *J. Gene Med.*, 2, 222–232, 2000.
95. De Smedt, S.C., Demeester, J., and Hennink, W.E., Cationic polymer-based gene delivery systems, *Pharm. Res.*, 17, 113–126, 2000.
96. Boussif, O., Lezoualc'h, F., Zanta, M., Mergny, M., Scherman, D., Demeneix, B., and Behr, J.P., A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: polyethylenimine, *Proc. Natl. Acad. Sci. USA*, 92, 7297–7301, 1995.
97. Van de Wetering, P., Cherng, J.Y., Talsma, H., and Hennink, W.E., Relation between transfection efficiency and cytotoxicity of poly(2-dimethylaminoethyl methacrylate)/plasmid complexes, *J. Control. Rel.*, 49, 59–69, 1997.
98. Walter, E., Dreher, D., Kok, M., Thiele, L., Kiama, S.G., Gehr, P., and Merkle, H.P., Hydrophilic poly(DL-lactide-co-glycolide) microspheres for the delivery of DNA to human-derived macrophages and dendritic cells, *J. Control. Rel.*, 76, 149–168, 2001.
99. Kimura, Y., Shirotani, K., Yamane, H., and Kitao, T., Copolymerization of 3-(S)-[(benzyloxycarbonyl)methyl]-1,4-dioxane-2,5-dione and L-lactide: a facile synthetic method for functionalized bioabsorbable polymer, *Polymer*, 34, 1741–1748, 1993.
100. Barrera, D.A., Zylstra, E., Lansbury, P.T., and Langer, R., Synthesis and RGD peptide modification of a new biodegradable copolymer: poly(lactic acid-co-lysine), *J. Am. Chem. Soc.*, 115, 11010–11011, 1993.
101. Lim, Y.B., Choi, Y.H., and Park, J.S., A self-destroying polycationic polymer: biodegradable poly(4-hydroxy-L-proline ester), *J. Am. Chem. Soc.*, 121, 5633–5639, 1999.
102. Putnam, D. and Langer, R., Poly(4-hydroxy-L-proline ester): low temperature condensation with plasmid DNA complexation, *Macromolecules*, 32, 3658–3662, 1999.

103. Lim, Y.B., Kim, C.H., Kim, K., Kim, S.W., and Park, J.S., Development of a safe gene delivery system using biodegradable polymer, poly([α-(4-aminobutyl)-L-glycolic acid], *J. Am. Chem. Soc.*, 122, 6524–6525, 2000.
104. Lim, Y.B., Han, S.O., Kong, H.U., Lee, Y., Park, J.S., Jeong, B., and Kim, S.W., Biodegradable polyester, poly([α-(4-aminobutyl)-L-glycolic acid], as a non-toxic gene carrier, *Pharm. Res.*, 17, 811–816, 2000.
105. Lee, M., Koh, J.J., Han, S.O., Ko, K.S., and Kim, S.W., Prevention of autoimmune insulitis by delivery of interleukin-4 plasmid using a soluble and biodegradable polymeric carrier, *Pharm. Res*, 19, 246–249, 2003.
106. Luten, J., Van Steenis, J.H., Van Someren, R., Kemmink, J., Schuurmans-Nieuwenbroek, N.M.E., Koning, G.A., Crommelin, D.J.A., Van Nostrum, C.F., and Hennink, W.E., Water-soluble cationic polyphosphazenes for gene delivery. *J. Control. Rel.*, 89, 483–497, 2003.

L2 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:218886 CAPLUS
DOCUMENT NUMBER: 139:312024
TITLE: Hydrogels by stereocomplex formation and their use as drug delivery matrices
AUTHOR(S): van Nostrum, Cornelus F.; de Jong, Sylvia J.; Kettenes-van den Bosch, Jantien J.; Hennink, Wim E.
CORPORATE SOURCE: Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, 3508 TB, Neth.
SOURCE: ACS Symposium Series (2003), 846(Advances in Controlled Drug Delivery), 129-141
CODEN: ACSMC8; ISSN: 0097-6156
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review. Block and graft copolymer hydrogels, phys. crosslinked through stereocomplex formation of poly- or oligo(lactic acid) chains of opposite chirality, are described. Protein-loaded hydrogels of dextran-g-oligo(L/D-lactic acid) are obtained from aqueous solns. of the two polymers (containing L- and D-grafts, resp.). These hydrogels degraded in a period of 1-7 days, depending on polymer composition Lysozyme was quant. released with full preservation of its activity.
REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

Chapter 10

Hydrogels by Stereocomplex Formation and Their Use as Drug Delivery Matrices

Cornelus F. van Nostrum[1], Sylvia J. de Jong[1,3], Jantien J. Kettenes-van den Bosch[2], and Wim E. Hennink[1]

Departments of [1]Pharmaceutics and [2]Biomedical Analysis, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, P.O. Box 80.082, 3508 TB Utrecht, The Netherlands
[3]Current address: Novartis Pharma BV, Raapopseweg 1, 6824 DP Arnhem, The Netherlands

Block and graft copolymer hydrogels, physically crosslinked through stereocomplex formation of poly- or oligo(lactic acid) chains of opposite chirality, are described. Protein-loaded hydrogels of dextran-*g*-oligo(L/D-lactic acid) are obtained from aqueous solutions of the two polymers (containing L- and D-grafts, respectively). These hydrogels degraded in a period of 1-7 days, depending on polymer composition. Lysozyme was quantitatively released with full preservation of its activity.

Introduction

Hydrogels are polymeric networks, which absorb and retain large amounts of water. In general, hydrogels possess a good biocompatibility. Their hydrophilic surface has a low interfacial free energy in contact with body fluids, which results in a low tendency for proteins and cells to adhere to these surfaces. Moreover, the soft and rubbery nature of hydrogels minimizes irritation to surrounding tissue. Therefore, hydrogels have found widespread application in different technological areas, e.g. as materials for contact lenses and protein separation, matrices for cell-encapsulation and devices for the controlled release of drugs and proteins.(*1*–*5*)

For many applications, such as drug delivery, it is advantageous that the hydrogels are biodegradable. Labile bonds can be present either in the polymer used to prepare the gel or in the crosslinks. These bonds can be broken under physiological conditions, in most of the cases by hydrolysis, either enzymatically or chemically.(*5*) It is of great interest to have control over the degradation kinetics; in other words, to have control over the parameters by which the degradation characteristics can be tailored. Moreover, once the hydrogels are implanted it is important that the formed degradation products have a low toxicity meaning that the formed compounds can either be metabolized into harmless products or can be excreted by the renal filtration process. The nature of the formed degradation products can be tailored by a rational and proper selection of the hydrogel building blocks.

Both chemical and physical methods have been used to create hydrogels.(6) In chemically crosslinked gels, covalent bonds are present between different polymer chains. In physically crosslinked gels, dissolution is prevented by physical interactions, which exist between different polymer chains. In recent years, there is an increasing interest in physically crosslinked gels, especially in which the gel formation occurs under mild conditions in the absence of organic solvents. The main reason is that the use of crosslinking agents and organic solvents to prepare such hydrogels is avoided. These agents and solvents can not only affect the integrity of the substances to be entrapped (e.g. proteins, cells) but they are often toxic compounds which have to be removed/extracted from the gels before they can be applied. To create physically crosslinked gels a wide variety of methods have been applied, including ionic, hydrophobic and hydrogen bond interactions.(6) Also the formation of crystalline domains is a tool to create physical crosslinks. The latter includes the formation degree of stereocomplexes, which is the subject of this contribution. In this contribution will describe our newly developed biodegradable hydrogel system based on biocompatible substances, *i.e.* dextran and lactic acid oligomers. These hydrogels can be prepared from pure aqueous solutions and can entrap and release proteins and enzymes without affecting their integrity.

Macromolecular Stereocomplexes

In low molecular weight compounds possessing a chiral center, the formation of racemic crystals upon mixing the two enantiomers is a well-known phenomenon. A higher melting temperature (T_m) is frequently observed for the racemic crystallites than for each of the enantiomers. For example, D- and L-tartaric acid have a T_m of 173 °C, whereas for its racemic mixture a T_m of 206 °C is detected.(*7*)

In polymers of opposite chirality the formation of racemic crystallites has also been observed. In the literature, the formation of such racemic crystallites has been referred to as stereocomplexes and was first described by Dumas *et al.* in 1972.(*8*) They reported a melting point of 165 °C for an optically active poly(*t*-butyl-thiirane) as compared to 205 °C for the corresponding blend of poly(*R*-*t*-butyl-thiirane) and poly(*S*-*t*-butyl-thiirane). The difference in melting temperature was due to different crystal structures, as reported by Matsubayashi *et al.*(*9*) Stereocomplexes were also observed for mixtures of the R- and S-forms of poly(α-methylbenzyl methacrylate),(*10*) poly(α-methyl-α-ethyl-β-propiolactone) (PMEPL),(*11*) poly(γ-benzyl glutamate),(12) poly(β-benzyl aspartate),(*13*) and α-olefin-carbon monoxide poly(1,4-ketone)s.(*14*)

It should be noted that the term stereocomplex is not exclusively used for racemic crystallites formed by chemically identical polymers of opposite chirality. Earlier, this term was used to describe the interaction between syndiotactic and isotactic polymers.(*15, 16*) Since these polymers do not have the same chemical structure, the term stereo-selective complexes, as suggested by Lohmeyer *et al.* is to be preferred in these cases rather than the term stereocomplexes.(*17*)

PLA Stereocomplex

Poly(lactic acid) (PLA) is a polyester, which consists of repeating units of lactic acid. Lactic acid, 2-hydroxypropionic acid, contains a chiral center and can therefore be in the L- or D-configuration. PLA is usually obtained by ring-opening polymerization of lactide, the cyclic dimer of lactic acid. Bulk polymerization of lactide with retention of stereochemistry can be carried out in the melt at 130 °C in the presence of the catalyst tin octoate.(*18, 19*) PLLA and PDLA, the homopolymers of L-lactic acid and D-lactic acid respectively, are semicrystalline materials. High molecular weight PLA, of either stereoisomer, has a melting temperature (T_m) of 170 °C, a melting enthalpy (ΔH_m) of 70 J/g, and a glass transition temperature (T_g) of 60 °C.(*20*) In blends of high molecular weight PDLA and PLLA, a phase with a higher T_m (230 °C) is observed. This phase is ascribed to stereocomplex formation. Racemic crystals were also

observed for the monomer lactide; the melting point (T_m) of D,D-lactide or L-lactide is 95 °C, whereas the 1:1 racemate of D,D-lactide and L,L-lactide has a higher melting point of 126 °C.(21) The ability of PLA to form stereocomplex was first described in a patent publication by Murdoch and Loomis,(22) and the first paper on these complexes was published by Ikada *et al.*(23)

By X-ray structure analysis, the unit cell of the crystals in the homopolymers of lactic acid was found to contain two 10_3 helices: poly(L-lactide) consists of left-handed helical chains and poly(D-lactide) of right-handed helical chains.(24) In the stereocomplex crystal, a poly(L-lactide) segment and a poly(D-lactide) segment are packed side by side in a 1:1 ratio of L and D monomer units and packed laterally in parallel fashion.(24) The unit cell of the complex contains three L- and three D-monomer units of the PLLA and PDLA helices, which can be packed more densely than left-handed or right-handed helices alone. Each PLLA and PDLA forms a more compact 3_1 helix in the complex crystal.(24–26) The following mechanism of growth for the triangular lamellar stereocomplex crystal was suggested by Brizzolara *et al.*:(27) As crystallization starts, for example, one PDLA helix will be surrounded by three PLLA helices. Because of the triangular shape of the 3_1 helix, a triangular nucleus is thus formed whose respective sides are built up exclusively by PLLA. In the next step a PDLA layer grows on the crystal structure and then again a PLLA layer grows onto the PDLA layer and so on. Van der Waals forces between the helices cause a specific energetic interaction-driven packing of the helices. These interactions cause the higher stability and consequently the higher melting point of the stereocomplex.(27)

PLA stereocomplexes were studied extensively as a new class of biodegradable materials with higher mechanical strength, improved thermal stability, and less sensitivity to hydrolysis than synthetic polyesters such as poly(glycolic acid) and PLA.(28) Stereocomplex PLA fibers were prepared by spinning from a mixed solution of PDLA and PLLA, and by dry spinning from a melt of PDLA and PLLA to obtain reinforced materials, which are stronger than PLLA.(29) However, PLA stereocomplexes have some drawbacks. They are highly resistant to degradation and thereby adversely affect the biocompatibility.(30–33) The poor biodegradation and biocompatibility of the PLA stereocomplexes resulted in a decreased interest in these systems. Nevertheless, at present stereocomplexes have regained interest for application in drug delivery systems.

Hydrogels Based on Stereocomplex Formation

Stereocomplex formation between PLLA and PDLA, as described above, has been applied by several groups for the preparation of biodegradable

hydrogels. The general feature of these hydrogels is that polymers or oligomers of either L-lactic acid or D-lactic acid are attached to a water-soluble polymer in the form of block or graft copolymers. Association takes place in crystalline domains (stereocomplexes) upon mixing the two polymers (one containing L-lactic acid, the other containing D-lactic acid), providing the physical crosslinks. We would like to classify PLA stereocomplex hydrogels as follows:

1. Hydrogels containing high molecular weight (HMW) PLA chains. The individual enantiomeric polymers (PLLA and PDLA) are already crystalline and mostly insoluble in water. Mixing should therefore take place from organic solutions or in the melt, and the resulting blend can subsequently be swollen in contact with water. The difference of these stereocomplex hydrogels with respect to hydrogels containing PLLA or PDLA alone is that the crystalline domains are more stable and resistant to hydrolytic degradation.
2. Hydrogels containing oligomeric lactic acid (OLA) chains, whose individual enantiomeric polymers (OLLA and ODLA) can be soluble in water when the lactic acid content is sufficiently low. This provides the unique opportunity to form hydrogels by stereocomplex crystallization from aqueous solutions.

Stereocomplex Hydrogels Containing HMW PLA.

Stereocomplex formation between triblock copolymers of PLLA-PEG-PLLA and PDLA-PEG-PDLA (PEG = poly(ethylene glycol)) was studied with the aim to prepare hydrogels.(*34, 35*) The release of bovine serum albumin (BSA) from microspheres based on these triblock copolymers, has been studied by Lim *et al.* and compared with the release of BSA from microspheres prepared with one enantiomeric form of the triblock copolymer and with PLA microspheres.(*34*) The protein-loaded microspheres were prepared by a double-emulsion solvent evaporation method. The stereocomplex and single enantiomeric triblock copolymer microspheres showed a slightly larger burst release than PLA microspheres, which is likely caused by the higher water-uptake capacity of the PEG-containing microspheres. Although the morphology of the stereocomplex microspheres was clearly deviating, the release of BSA was similar to the single enantiomeric triblock copolymer microspheres.

Stereocomplex Hydrogels Containing OLA.

The triblock copolymers mentioned in the previous section are water-soluble when the hydrophobic PLA blocks are sufficiently short. The maximum

length of the lactic acid blocks for rendering water-solubility decreases with decreasing length of the PEG blocks used. For example, PEG-block with a molecular weight of 13.000 g mol^{-1} substituted with on average 26 lactic acid repeating units on each side of the PEG block are water-soluble and can form stereocomplexes upon mixing of aqueous solutions of each enantiomer.(*35*)

Recently, another system has been prepared by Lim *et al.*, based on stereocomplex formation by enantiomeric oligo(lactic acid) (OLA) side chains grafted onto poly(2-hydroxyethyl methacrylate) (PHEMA) (poly(HEMA-*g*-oligolactate)s).(*36*) The system was prepared by casting a film from poly(HEMA-*g*-oligo(L)lactate) and poly(HEMA-*g*-oligo(D)lactate), both dissolved in chloroform. Among other characteristics, the degradation of the obtained film was compared with the degradation of a film cast from a solution of a single enantiomer of the graft copolymer. Slower degradation was observed for the 1:1 blend of the L- and D-forms than for the single enantiomer. Stereocomplex formation from water was not investigated and is most likely not possible due to the high grafting density.

We realized the importance of avoiding organic solvents for the dissolution of the individual enantiomeric polymers to be used for the formation of stereocomplex hydrogels when aiming at biomedical applications such as the delivery of pharmaceutically active proteins. Therefore, we prepared biodegradable and biocompatible hydrogels based on dextran (a natural occurring polysaccharide) grafted with OLA and investigated the minimum and maximum length of the grafts required to form stereocomplexes after mixing and retaining water-solubility before mixing, respectively. The results and the application as a protein delivery device will be summarized in the next section.

Dextran-*g*-OLA Stereocomplex Hydrogels

In our Department we designed a hydrogel system based on dextran in which crosslinking is established by stereocomplex formation between lactic acid oligomers of opposite chirality. First, we investigated whether an 'operation window' of lactic acid chain lengths is present, in which stereocomplex crystallization would occur whithout homocrystallization of the individual enantiomers. Therefore, we isolated monodisperse lactic acid oligomers by preparative HPLC, from a polydisperse mixture obtained by conventional ring opening polymerization of L- or D-lactide. It was shown that crystallinity was present in individual D- or L-oligomers with a degree of polymerization (DP, *i.e.* the number of lactic acid repeating units) ≥ 11. On the other hand, in blends of D- and L-oligomers of lactic acid crystallinity (stereocomplexation) was already observed at a DP ≥ 7 (see Figure 1).(*37*)

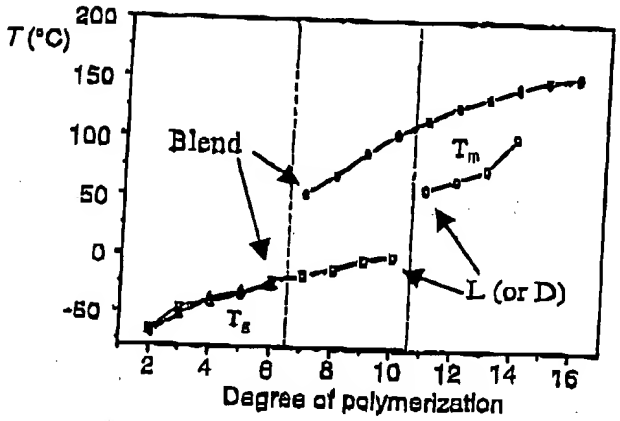


Figure 1. Glass transition (T_g) and melting temperatures (T_m) of lactic acid oligomers from differential scanning calorimetry measurements: OLLA (or ODLA) (open symbols) and 1:1 mixture of OLLA and ODLA (filled symbols). (Adapted with permission from reference 37. Copyright 1998 American Chemical Society.)

In the next step, polydisperse or monodisperse L- and D-lactic acid oligomers were coupled via their terminal hydroxyl group to dextran, yielding dextran-*g*-OLLA and dextran-*g*-ODLA, respectively, with variation in DP of the oligolactic acid and degree of substitution (DS, percentage of substituted dextran repeating units) (Figure 2). Interestingly, each product was soluble in water separately and upon mixing solutions containing OLLA- and ODLA-grafted dextran, hydrogels are formed at room temperature as demonstrated by rheological measurements.(*38*) As can be seen in Figure 3, the storage modulus of the obtained hydrogel strongly decreased upon heating to 80 °C, while it was restored upon cooling to 20 °C demonstrating the thermo-reversibility and the physical nature of the crosslinks. The storage modulus of the gels depends on the degree of polymerization of the lactate acid grafts and their degree of substitution on dextran. Mixtures of dextran-*g*-OLLA and dextran-*g*-ODLA containing monodisperse grafts with a DP lower than 11 did not result in a hydrogel. This is in contrast to the observation that stereocomplexation already can occur for non-grafted OLA chains with a DP ≥ 7. This difference can be explained by hampered stereocomplex formation once the oligomers are both coupled via their hydroxyl group to dextran (Figure 4A). Interestingly, gel formation was favored when one lactic acid oligomer was coupled via its

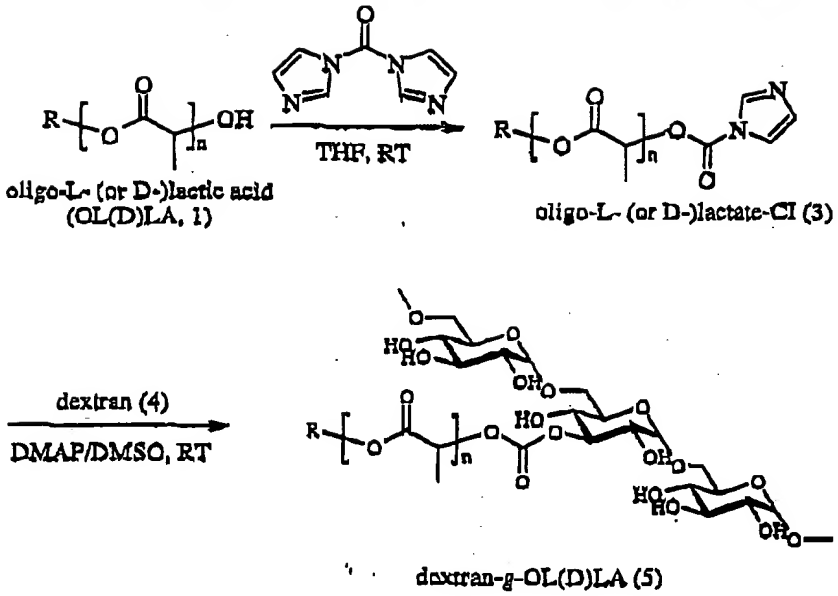


Figure 2. Synthesis of dextran-g-OLA. R represents a 2-(2-methoxy-ethoxy)ethyl (MEE) group.

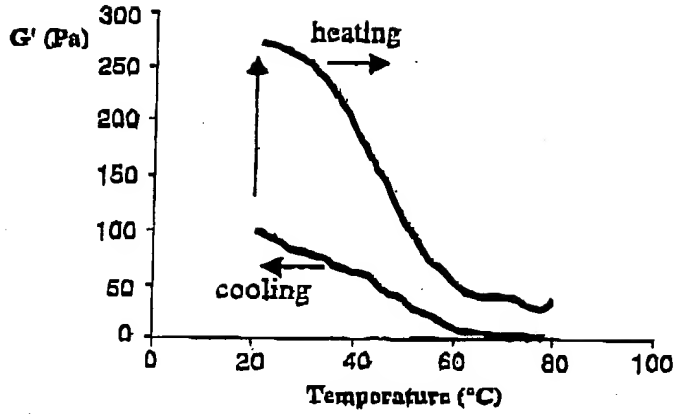


Figure 3. Storage modulus as a function of temperature of a dextran-g-OLA stereocomplex hydrogel ($DP_{average}$ = 9, DS = 3, 80 % water) upon heating and cooling. The vertical arrow reflects the increase in storage modulus in time at 20 °C to its original value. (Adapted from reference 38.)

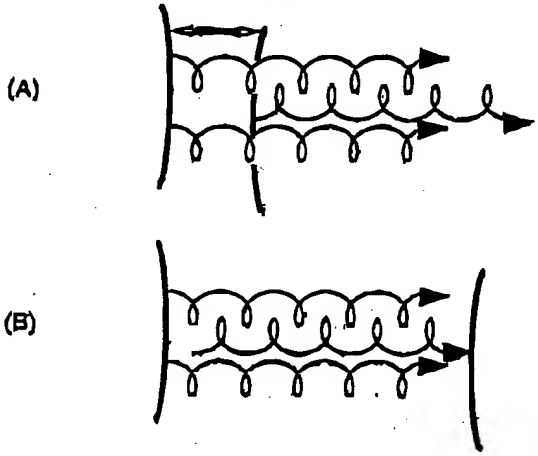


Figure 4. Schematic representation of stereocomplex formation in dextran-g-OLA hydrogels, showing the required unidirectional packing of the lactic acid chains in two cases: with both enantiomeric OLA chains connected to dextran via their OH terminus giving steric hindrance between the dextran chains (A) and one of either enantiomers coupled via the OH terminus and the other via its carboxylate terminus (B). The carboxylate termini are represented by the black arrowheads.

hydroxyl group whereas the oligomer of opposite chirality was coupled via its carboxylic acid group.(*39*) This is ascribed to the required parallel packing of the oligomers in stereocomplexes, *i.e.* in which all the chains are oriented in the same direction (as explained in Figure 4).(*24*)

Protein-loaded hydrogels were simply prepared by dissolving the protein in the dextran-g-OLLA/ODLA solutions prior to mixing. It was shown that under physiological conditions the gels are fully degradable.(*40*) The degradation time depended on the pH and the composition of the hydrogel, *i.e.* the number of lactate grafts, the length and polydispersity of the grafts and the initial water content, and varied from 1 to 7 days (Figure 5). Under non-degrading conditions (pH 4) the hydrogels, having a water content of almost 90% in their swollen states, appeared to be stable for more than 1 month. As shown in Figure 6, the gels showed a release of the entrapped model proteins (IgG and lysozyme) over 6 days and the kinetics depended on the gel characteristics, such as the polydispersity of the lactate grafts and the initial water content. The release of lysozyme was by diffusion, whereas for the bigger IgG, whose hydrodynamic radius approaches the estimated mesh size of the hydrogels, also swelling/degradation played a role in the release. Importantly, the proteins were quantitatively released from the gels and with full preservation of the enzymatic activity of lysozyme, emphasizing the protein-friendly preparation method of the protein-loaded stereocomplex hydrogel.

Conclusions

Hydrogels which are physically crosslinked by stereocomplex interactions have attracted recent attention for drug delivery purposes. Especially systems which are obtained from aqueous solutions of the two components are very attractive, since they provide a friendly environment for the encapsulation of highly sensitive bioactive substances (proteins, DNA, living cells). Moreover, it is anticipated that gel formation can take place *in situ* after injection of the low-viscous solutions. We have developed a versatile and fully degradable system obtained from water soluble dextran grafted with oligolactic acid chains. The mechanical properties, degradation profile and release of encapsulated compounds can be simply tailored by the composition of the materials. At present we are investigating means to extend the degradation time by changing the chemistry of the bonds between the grafts and the backbone. Also, the preparation of injectable microspheres is one of our goals. The biocompatibility of the system will be established, but no problems are expected in that respect since recent *in vivo* studies on chemically crosslinked dextran hydrogels already showed good biocompatibility.(*41*)

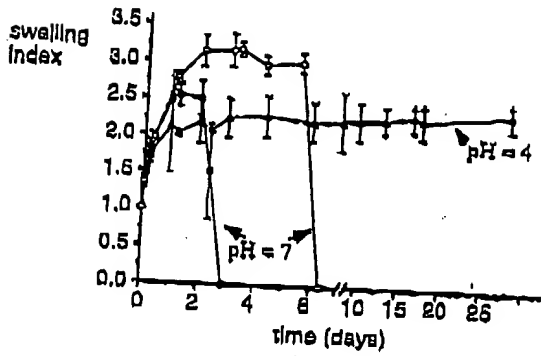


Figure 5. Swelling behavior of dextran-g-OLA stereocomplex hydrogels (DS = 6, 70 % water, 37 °C): high polydispersity lactic acid grafts ($DP_{average}$ = 12, M_w/M_n = 1.25, filled symbols) and low polydispersity grafts (DP = 11 to 14, M_w/M_n = 1.01, open circles). The filled triangles represent swelling under non-degrading conditions (pH 4) (average ± s.d., n = 3 or 4). (Adapted with permission from reference 40. Copyright 2001 Elsevier Science B.V.)

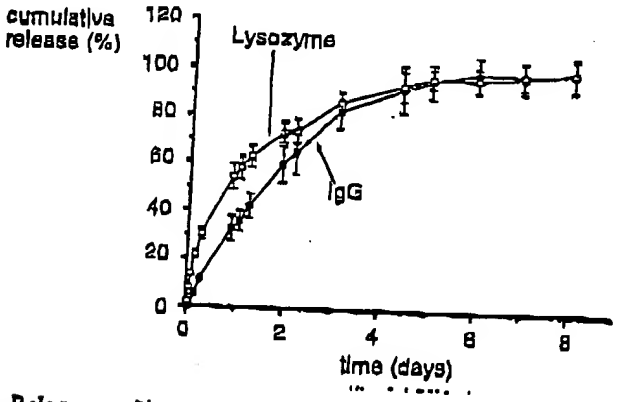


Figure 6. Release profiles of lysozyme (open squares) and IgG (filled circles) from dextran-g-OLA stereocomplex hydrogel with low polydispersity grafts (DS = 6, DP = 11 to 14, 70 % water, pH 7, 37 °C) (average ± s.d., n = 4). (Adapted with permission from reference 40. Copyright 2001 Elsevier Science B.V.)

References

1. *Hydrogels in medicine and pharmacy;* vol. I, II, III; Peppas, N. A., Ed.; CRC Press: Boca Raton, 1986.
2. Peppas, N, A.; Bures, P.; Leobandung, W.; Ichikawa, H. *Eur. J. Pharm. Biopharm.* 2000, *50*, 27-46.
3. Peppas, N. A.; Huang, Y.; Torres-Lugo, M.; Ward, J. H.; Zhang, J. *Annu. Rev. Biomed. Eng.* 2000, *2*, 9-29.
4. Gehrke, S. H. *Drugs Pharm. Sci.* 2000, *102*, 473-546.
5. *Biodegradable hydrogels for drug delivery;* Park, K.; Shalaby, W. S. W.; Park H., Eds.; Technomic Publishing Company: Basel, Switzerland, 1993.
6. Hennink, W. E.; van Nostrum, C. F. *Adv. Drug Delivery Rev.* 2002, *54*, 13-36.
7. Parry, G. S. *Acta Cryst.* 1951, *4*, 131-138.
8. Dumas, P.; Spassky, N.; Sigwalt, P. *Makromol. Chem.* 1972, *156*, 55.
9. Matsubayashi, H.; Chatani, Y.; Tadokoro, H.; Dumas, P.; Spassky, N.; Sigwalt, P. *Macromolecules* 1977, *10*, 996-1002.
10. Hatada, K.; Shimizu, S.-I.; Terawaki, Y.; Ohta, K.; Yuki, H. *Polym. J.* 1981, *13*, 811-812.
11. Grenier, D.; Prud'homme, R. E. *J. Polym. Sci., Polym. Phys. Ed.* 1984, *22*, 577-587.
12. Fukuzawa, T.; Uematsu, I. *Polym. J.* 1974, *6*, 537.
13. Sakajiri, K.; Kawasaki, E.; Watanabe, J. *Macromolecules* 2001, *34*, 7238-7240.
14. Jiang, Z.; Boyer, M. T.; Sen, A. *J. Am. Chem. Soc.* 1995, *117*, 7037-7038.
15. Watanabe, W. H.; Ryan, C. F.; Fleisher Jr, P. C.; Garret, B. S. *J. Phys. Chem.* 1961, *65*, 896.
16. Liquori, A. M.; Anzuino, G.; Corio, V. M.; D'Alagni, M.; DeSantis, P.; Savino, M. *Nature* 1965, *206*, 358-362.
17. Lohmeyer, J. H. G. M.; Tan, Y. Y.; Loka, P.; Challa, G. *Polymer* 1978, *19*, 1171-1175.
18. Krisheldorf. H. R.; Kreiser-Saunders, I.; Boettcher, C. *Polymer* 1995, *36*, 1253-1259.
19. Witzke, D. R.; Narayan, R.; Kolstad, J. J. *Macromolecules* 1997, *30*, 7075-7085.
20. Jamshidi, K.; Hyon, S. H.; Ikada, Y. *Polymer* 1988, *29*, 2229-2234.
21. van Hummel, G. J.; Harkema, S.; Kohn, F. E.; Feijen, J. *Acta Crystallogr.* 1982, *B38*, 1679-1681.
22. Murdoch, J. R.; Loomis, G. L. U.S. Patent 4,719,246, 1988.
23. Ikada, Y.; Jamshidi, K.; Tsuji, H.; Hyon, S.-H. *Macromolecules* 1987, *20*, 904-906.
24. Oldhera, T.; Tsuji, M.; Kawaguchi, A.; Katayama, K. *J. Macromol. Sci., Phys.* 1991, *B30*, 119-140.

25. Loomis, G.; Murdoch, J.; Gardener, K. *Polym. Prep.* **1990**, *31*, 55.
26. Spinu, M.; Gardener, K. H. *Polym. Mat. Sci. Eng.* **1994**, *74*, 19-20.
27. Brizzolara, D.; Cantow, H.-J.; Diederichs, K.; Keller, E.; Domb, A. *Macromolecules* **1996**, *29*, 191-197.
28. Tsuji, H.; Ikada, Y. *Polymer* **1999**, *40*, 6699-6708.
29. Tsuji, H.; Ikada, Y.; Hyon, S.; Kimura, Y.; Kitao, T. *J. Appl. Polym. Sci.* **1994**, *51*, 337-344.
30. Li, S.; Vert, M. *Macromolecules* **1994**, *27*, 3107-3110.
31. Vert, M.; Mauduit, J.; Li, S. *Biomaterials* **1994**, *15*, 1209-1213.
32. Bergsma, E. J.; Rozema, F. R.; Bos, R. R. M.; de Bruyn, W. C. *J. Oral Maxillofac. Surg.* **1993**, *51*, 666-670.
33. Li, S.; Vert, M. *Polym. Int.* **1994**, *33*, 37-41.
34. Lim, D. W.; Park, T. G. *J. Appl. Polym. Sci.* **2000**, *75*, 1615-1623.
35. Grijpma, D. W.; Feijen, J. *6th Eur. Symp. Controlled Drug Delivery* **2000**, 113-115.
36. Lim, D. W.; Choi, S. H.; Park, T. G. *Macromol. Rapid Commun.* **2000**, *21*, 464-471.
37. de Jong, S. J.; van Dijk-Wolthuis, W. N. E.; Kettenes-van den Bosch, J. J.; Schuyl, P. J. W.; Hennink, W. E. *Macromolecules* **1998**, *31*, 6397-6402.
38. de Jong, S. J.; de Smedt, S. C.; Wahls, M. W. C.; Demeester, J.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *Macromolecules* **2000**, *33*, 3680-3686.
39. de Jong, S. J.; de Smedt, S. C.; Demeester, J.; van Nostrum, C. F.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *J. Controlled Release* **2001**, *72*, 47-56.
40. de Jong, S. J.; van Eerdenbrugh, B.; van Nostrum, C. F.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *J. Controlled Release* **2001**, *71*, 261-275.
41. Cadée, J. A.; van Luyn, M. J. A.; Brouwer, L. A.; Plantinga, J. A.; van Wachem, P. B.; de Groot, C. J.; den Otter, W.; Hennink, W. E. *J. Biomed. Mater. Res.* **2000**, *50*, 397-404.